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CANCER RESEARCH

A MONTHLY JOURNAL OF ARTICLES AND ABSTRACTS REPORTING CANCER RESEARCH

VOLUME 5 OCTOBER, 1945 NUMBER 10

A System of Grading Carcinogenic Potency*

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(Received for publication April 10, 1945)

It is frequently necessary to evaluate carcinogenic potency and neoplastic response on a quantitative basis. In theory, potency and response refer to the same thing, looked at from different angles: potency, from the point of view of the carcinogen; response, from the point of view of the test animal. But the methods of estimating the two are not identical in practice.

The evaluation of differences in response usually requires statistical analyses of large-scale experiments. From recent studies by Fieser (1), Bryan and Shimkin (2, 3), and others, a quantitative relation has been established between the dose of a carcinogen injected subcutaneously and the neoplastic response, and a careful assessment of different methods has demonstrated the greater reliability of estimating response in terms of *tumor incidence at different dose levels* than in terms of *average induction period*. With the help of these refinements in technic, it is now possible to determine with great accuracy any influence that biological or other variables might exert on the neoplastic response of a group of animals under standard conditions.

But for a general assessment of *carcinogenic potency* the exacting demands of large-scale experiments at different dose levels are impracticable, while the emphasis on minute differences, arising from such precise methods, can be more of a hindrance than a help. Furthermore, the refinements mentioned above are primarily designed for the better planning of controlled experiments of the future, whereas a system of grading carcinogenic potency must of necessity be applicable in retrospect to inadequate data. There are, for instance, the hundreds of pure chemical compounds that have already been examined for carcinogenic action (4). These were mainly tested on small groups of animals, using only one concentration in most cases, and many of these compounds may never be re-tested on a larger scale. Such data are nevertheless of great value, and obviously cannot be ignored in any proposed system of grading carcinogenic potency; yet they are unsuitable for statistical analysis, or for assessment by any method based on dose-response relationship.

Hence an acceptable system of grading carcinogenic potency must be based on principles that are very different from those required for the more detailed evaluation of neoplastic response. The method must be simple, to have a reasonable chance of universal acceptance; its aim must be towards approximation rather than precision; it must be applicable to the weakest as well as the strongest carcinogens; and, in order that previously recorded results should not be wasted, it must be sufficiently flexible to allow of alternative presentations of the data.

DETAILS OF THE PROPOSED SYSTEM OF GRADING

In trying to correlate data obtained under varying experimental conditions, difficulties at once arise through (a) differences in the species of animals used and the site and methods of administration of the carcinogen; and (b) differences in the amounts of carcinogen administered and the frequency of application or injection.

With regard to the first of these, no compromise or approximation is possible. Whatever the method of grading adopted, it will be necessary to specify in every case the species of animal and the site of administration to which the grading refers. A compound may be found to be of one grade for subcutaneous injection in the rat, of another grade for subcutaneous injection in the mouse, and of yet another grade for skin application in the mouse. It is also desirable to specify whether the grading for skin application refers to both papilloma and carcinoma, or to carcinoma only.

But with the problem of varying dosage and frequency of administration the difficulties are not so serious, and can be largely overcome. In the case of

* Because of the difficulties of international communication the author has not read proof of this article.

subcutaneous injection, the dose-response relationship holds only for very low concentrations (2, 3), since above a certain dose level (which may be called the "saturation dose") the neoplastic response varies but slightly over a wide range of concentration. Since this "saturation dose" is of the order of 1 mgm., and as most tests for carcinogenesis by the subcutaneous route are made with doses larger than this amount, the question of varying dosage becomes relatively unimportant, especially in a system of grading which, like the present one, does not claim to be too exacting. Similarly, in the case of skin application, it is generally agreed that variations in the concentration of the carcinogen within the limits of 0.3 to 1.0 per cent have relatively little effect on the neoplastic response, and that differences in response due to variations in the frequency of application are also not great, provided the frequency is not less than once a week or more than three times a week (5, 6). Fortunately, skin tests are usually carried out within these ranges.

With the elimination of the factor of dosage in this manner, the only suitable method of evaluation available is that of the *latent period of carcinogenesis.* For preference, this must refer to some mean value for the whole group of animals tested, though considerable latitude is essential, as almost every investigator presents his results in a slightly different form. For skin painting, a satisfactory criterion is *the time taken for 50 per cent of the surviving animals to develop tumors.* A useful variant of this, and one that provides results not very different, is to estimate the 50 per cent level on the basis of the *effectual total* of animals (*i. e.,* on the number that survived the period for the first tumor to arise in that series). This method is even to be preferred in the case of subcutaneous injection, since early tumor-bearers are likely to die before the late ones begin to respond. However, it is found that published results often do not provide sufficient data for either method to be employed. In such cases the *average latent period* may be used instead, and will yield a figure similar to the 50 per cent level provided the total tumor yield of the series in question is high (*i. e.,* approaching 100 per cent). Obviously, the lower the total tumor yield, the less representative the "average latent period" becomes, since in the extreme case this "average" becomes synonymous with the time of appearance of the first (and only) tumor.

In brief, it is proposed to base the present system of grading on the 50 per cent level, or its equivalent. But one must still consider the case of very weak carcinogens that never yield 50 per cent of tumors. For these some approximation is necessary, to bring them into line with the others.

Perhaps the simplest method is that based on the personal observation of the author, that with the more active compounds the time taken for 50 per cent of tumors to develop is usually about twice as long as that taken for the first tumor to appear in the series. (To quote some examples from the author's own results from skin painting experiments on mice:—16 and 8 weeks respectively for 3,4-benzpyrene; 33 and 18 weeks respectively for 1,2,5,6-dibenzanthracene. The same applies to many of the published data in the literature.) Assuming that a similar approximate relationship is valid for weaker agents, it is possible to include the data for them, by taking the time of appearance of the first tumor, multiplying it by two, and accepting the product as representing *approximately* what the 50 per cent level would have been if the animals had lived long enough. But as the values obtained by this method are obviously less accurate than those obtained by the standard method, it is proposed to distinguish between the two by expressing the less accurate assessment in Arabic instead of Roman numbering (*e. g.,* Grade 3, as contrasted with Grade III).

We now come to the actual method of grading, calculated on the basis of the 50 per cent level or its equivalent. Twelve grades of potency are chosen, since a larger number would assume a degree of accuracy and precision that the system does not, in fact, possess, while a smaller number would make the system too insensitive. In order that it may cover the whole range of potency the highest grade (XII) must at least correspond to the induction period of the most potent carcinogen known, acting under optimal conditions, while the lowest grade (I) must correspond to the weakest agent, which, in terms of induction time, means the life span of the test animals likely to be used.

A suitable range is from 4 to 200 weeks. Since a linear relationship between induction time and grading would give undue prominence to the weakest agents (for which, in any case, least accuracy can be expected), the time (50 per cent level) is plotted *on a logarithmic scale* against the 12 grades plotted on an ordinary scale, and a straight line is drawn to join the points of intersection of Grade XII at 4 weeks, with Grade I at 200 weeks (Fig. 1). For convenience, an additional scale in months, instead of weeks, is also provided. Once the 50 per cent level (or its equivalent) is known, the appropriate grade can be read off from the curve. Alternatively, the grade can be calculated from the formula corresponding to the curve (see Appendix I).

Where a choice of data is available from the literature, greatest reliance should naturally be given to results of experiments carried out on large numbers of animals, and involving a low mortality. If the

values of separate tests with the same compound vary considerably (owing to differences in strains of animals, etc.) it is advisable to exclude extremes, if representative grading is to be achieved. The system is too insensitive to permit the use of fractions of grades, so that when such fractions are obtained from the curve (or formula) the nearest whole number must be

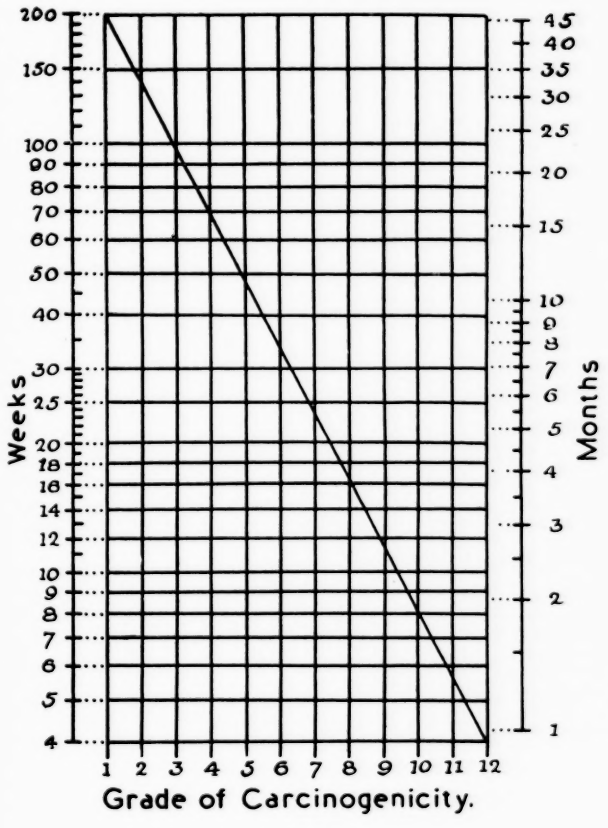

FIG. 1

chosen. A *slight* degree of weighting may be permissible in special circumstances. Inferences for *grading up* would be when the dose of carcinogen employed was unusually small, or when ulceration after a single injection suggests loss of the bulk of the injected material. Inferences for *grading down* would be when exceptionally large and frequently repeated doses were used, and also when the results, expressed in the form of "average latent period," were based on experiments in which the total tumor yield was much lower than 100 per cent. Such minor adjustments must be made with caution, lest the very basis of a universal system of grading—standardization—be jeopardized.

When the data provided are not suitable for precise assessment, an approximate grading is preferable to no grading at all, provided the approximation is made clear by some sign (*e. g.,* by a question mark following the grade number). Of course, where the published data give only the percentage total of tumors, without any reference to time, no assessment by the present system is possible. But such cases are exceptional. Difficulty also arises when the figures given refer to the times when the tumor-bearing animals died, and not when the tumors were first observed. In such cases, the grading should be based on the figures provided, but the grade number should then be followed by a plus sign, to indicate that in all probability the assessment is too low. Such provisional grading might later be corrected by the original authors after consulting their unpublished protocols. On the other hand, where there is a strong suspicion that the assessment is too high, a minus sign after the grade number might indicate this fact.

Except for these minor adjustments, the present system of grading should be applicable to most of the available published data, so that the results of various authors, using different methods of administration and recording, could be compared in a relatively simple manner.

APPENDIX I

The formula corresponding to the curve in Fig. 1 may be expressed as follows:

$$G = 16 - 6.5(\log W); \text{ or } G = 12 - 6.5(\log M);$$

where G = the grade (to be expressed as the nearest whole number in roman numerals);
W = latent period in weeks (average, or 50 per cent level)
M = latent period in months (" " " " " ")

In the case of very weak carcinogens; the appropriate formula would be:

$$g = 16 - 6.5(\log 2w); \text{ or } g = 12 - 6.5(\log 2m);$$

where w and m represent the times of appearance of the first tumor (in weeks and months respectively); while the value g represents the grade (to be expressed in arabic numerals).

APPENDIX II

Examples Chosen to Illustrate the Grading System

Carcinogen	Grading for mice			
	Papilloma and carcinoma (skin application)		Sarcoma (subcutaneous injection)	
	Grade	Reference*	Grade	Reference*
9, 10-dimethyl-1, 2-benzanthracene	X	Bachmann *et al.* (7)	VII	Shear *et al.* (8)
20-methylcholanthrene	VIII	Fieser (1)	IX	Fieser (1)
3, 4-benzpyrene	VIII	Fieser (1)	VIII	Fieser (1)
cholanthrene	VII	Bachmann *et al.* (9)	IX	Shear (10)
3, 4, 5, 6-dibenzcarbazole	VII	Boyland and Brues (11)	IX	Strong *et al.* (12)
2-methyl-3, 4-benzphenanthrene	VII	Bachmann *et al.* (9)	5+	Badger *et al.* (13)
10-methyl-1, 2-benzanthracene	VI?	Badger *et al.* (13)	VIII+	Shear (14)
1, 2, 5, 6-dibenzanthracene	VI	Fieser (1)	VII	Fieser (1)
5, 6-dimenthyl-1, 2-benzanthracene	VI	Barry *et al.* (15)	.....	.....
5-methyl-1, 2-benzanthracene	V	Bachmann *et al.* (9)	VI	Shear (14)
1, 2, 5, 6-dibenzcarbazole	5	Boyland and Brues (11)	0	Badger *et al.* (16)
3, 4-benzphenanthrene	V	Barry *et al.* (15)	0	Badger *et al.* (13)
1, 2, 5, 6-dibenzacridine	4	Barry *et al.* (15)	IV+	Badger *et al.* (13)
3, 4, 5, 6-dibenzacridine	IV	Barry *et al.* (15)	2+	Badger *et al.* (13)
1, 2-benzanthracene	1+	Barry *et al.* (15)	0	Barry *et al.* (15)

* The references given above refer to the publications containing the data from which the grading has been calculated. In the case of the three compounds (methylcholanthrene, benzpyrene, and dibenzanthracene) most extensively tested, the grading has been calculated from the most reliable data summarized by Fieser (1).

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The Effect of the Autoxidizability of Lipoidal Solvents on Sarcogenesis by 3,4-Benzpyrene*

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It is now well known that the rate of formation and the final incidence of sarcomas produced by the subcutaneous injection of a hydrocarbon are influenced in a conspicuous manner by the solvent in which the carcinogen is dissolved (11). Because of invariable exceptions, attempts to classify lipids according to their effect on tumor formation have not been very successful. Nevertheless, the existing data suggest that oils from plant sources augment sarcogenesis while a number of lipids from animals retard it (1, 5, 9). No satisfactory interpretation for these differences has been offered.

Fats obtained from plant or animal sources differ in many respects, but one variation worthy of investigation is their dissimilarity in lability to autoxidation. Most vegetable oils contain considerable amounts of antioxidants and their stability to autoxidation is well known, whereas animal fats (lard) contain only small amounts of inhibitols and are rather susceptible to oxidation (8). Previous publications from our laboratory have shown that both 3,4-benzpyrene and 20-methylcholanthrene undergo a coupled oxidation in the presence of autoxidizing fats but are stable in non-oxidizing lipids (6, 7). It is possible, therefore, that the low incidence of tumors that occurs when certain animal fats are used as solvents may be due to oxidative destruction of the carcinogen before it can exert its effect on the tissues.

To test the effect of the autoxidizability of lipoidal solvents on sarcogenesis, 3,4-benzpyrene was added to several types of fatty substances that possessed varying degrees of stability to autoxidation, and the sarcogenic activities of the mixtures were tested in mice. In some experiments the antioxidants were removed from the vehicle and in other cases they were added to the solvent. Finally, the rate of destruction of 3,4-benzpyrene in various solvents was determined both *in vitro* and *in vivo*, and these findings were compared to the rate of tumor formation observed in parallel experiments.

* This investigation was aided by a grant from the Jonathan Bowman Fund for Cancer Research.

MATERIALS AND METHODS

Ethyl linolate was prepared by the method of Rollett (10), and further purified by distillation *in vacuo*. Tricaprylin was prepared according to the directions of Hartwell (2). Methyl caprate, ethyl palmitate, ethyl stearate, and ethyl oleate were highly purified preparations kindly furnished by Dr. Schuette, of the Chemistry Department. Cottonseed oil (Wesson oil) and corn oil (mazola) were commercial products. Cottonseed oil was largely freed from antioxidants by passing a solution of the oil in Skelly solve B through separate columns of activated alumina and neutrol-5 (14). The lard filtrate and lard residue were prepared according to the directions of Leiter and Shear (3). A good quality of leaf lard was filtered at 38° for 24 to 36 hours through coarse filter paper. The fraction that was liquid at 38° and passed through the paper was designated "lard filtrate," and the material remaining on the filter paper was designated "lard residue." A crude fraction of lecithin was prepared from fresh rat liver by covering the tissue with 2 volumes of acetone, mincing in a Waring blendor, centrifuging, and re-extracting with a 1:4 alcohol-ether mixture. The solvent was evaporated under reduced pressure, and the residue taken up in a small volume of ether and poured into acetone. Further purification of the lecithin was obtained by twice reprecipitating it with acetone from a solution of ether. Mouse fat was prepared by two methods. In one experiment, minced eviscerated mouse carcasses were dried thoroughly with acetone and extracted with ethyl ether in a Soxhlet apparatus for 24 hours. A dark brown semisolid fatty substance remained after evaporation of the ether. For another experiment mouse fat was extracted from minced eviscerated mouse carcasses with Skelly solve B in a Waring blendor; the crude oil remaining after the evaporation of the solvent *in vacuo* was lighter both in color and in consistency than the material obtained by the first method.

The 3,4-benzpyrene was a purified crystalline substance. The α-tocopherol was a pure product obtained

through the courtesy of Dr. D. F. Robertson, of Merck and Company.

Albino mice, either of strain C or of the mixed ABC stock were used, and in most cases 40 mice equally divided between sexes were employed in each group. The mice were from 3 to 4 months of age at the beginning of the experiments, and were kept on pine shavings in metal boxes. A stock diet of Friskies dog biscuit and water was freely available to all groups at all times except for one series fed a semisynthetic diet (12), in which the mice were limited to 9.7 calories per mouse per day.

The mice were given a single subcutaneous injection of 0.1 or 0.2 cc. of lipid, containing 0.1 or 0.2 mgm. respectively of 3,4-benzpyrene in solution. The lard residue was heated to about 40° in order to facilitate the injections. The animals were examined by palpation every 2 weeks for progressively growing masses.

In order to determine the rate of disappearance of 3,4-benzpyrene in various lipids, samples of the hydrocarbon-lipid mixtures were set aside in test tubes and examined at regular intervals for the presence of benzpyrene according to methods previously described (4). Furthermore, additional mice were injected with each mixture, 5 from each group were sacrificed at intervals, and the amount of benzpyrene remaining in the subcutaneous tissue was determined (4). Great care was used for the removal of all the carcinogen from the subcutaneous tissue, since in some cases it was encapsulated in a thin-walled cyst that broke easily; in some animals the benzpyrene had spread over a large area, while in others it had become thick and cheese-like in consistency. Because of such variations, the skin and subcutaneous tissue were excised from a wide area surrounding the site of injection. The excisions were performed under a source of ultraviolet light; in this way the fluorescence of the remaining hydrocarbon determined the extent of the area to be excised.

RESULTS

The influence of antioxidants on fats of plant or animal origin.—To determine whether the difference in the incidence of tumors obtained with some fats of animal origin as compared with plant fats was due to their respective differences in antioxidant content, the effect of the following fats on tumor formation was tested: (a) cottonseed oil largely freed of antioxidants; (b) the same cottonseed oil as used in (a), but with the addition of α-tocopherol; (c) mouse fat obtained from dried mouse carcasses; and (d) the same as (c), but containing an added amount of α-tocopherol. Each mouse received a single subcutaneous injection of 0.2 mgm. of 3,4-benzpyrene dissolved in 0.2 cc. of one of the oils. The results of this experiment are shown in Table I. The addition of α-tocopherol to cottonseed oil accelerated the rate of tumor formation and increased the final incidence of neoplasms. Thus by 4 months 83 per cent of the mice injected with benzpyrene containing tocopherol had sarcomas as compared to an incidence of 38 per cent for the control group, and by 6 months the number of tumors for the 2 groups had increased to 98 per cent and 75 per cent respectively. It is possible that the spread in tumor incidence between the 2 groups might have been even more conspicuous if the level of carcinogen injected had been just one-half the amount given.

TABLE I: THE EFFECT OF THE ANTIOXIDANT CONTENT OF COTTONSEED OIL AND MOUSE FAT ON SARCOGENESIS

(Each mouse received a single subcutaneous injection of 0.2 cc. of oil containing 0.2 mgm. of 3,4-benzpyrene. Strain C mice were used.)

Lipid vehicle	Number of mice (effectual total)	Cumulative tumor count: After injection of benzpyrene, months 3	4	5	6	Number of mice tumor-free and alive at end of experiment	Tumors based on effectual total, per cent
Cottonseed oil *	40	5	15	25	30	10	75
Cottonseed oil * (containing 0.09 mgm. of α-tocopherol)	40	6	34	39	39	0	98
Mouse fat **	39	3	16	18	24	15	62
Mouse fat ** (containing 0.09 mgm. of α-tocopherol)	40	5	20	23	25	15	63

* Cottonseed oil largely freed of antioxidants.

** Mouse fat obtained by the extraction of dried mouse carcasses.

The addition of tocopherol to mouse fat prepared by extraction of the dry carcass had no influence either on the rate of tumor appearance or on the final incidence of sarcomas (Table I). The number of neoplasms developing in these groups, however, was considerably higher than that reported by other investigators using mouse fat (1, 5, 9). Our preparation was obtained by a thorough extraction of dried mouse carcasses, whereas the workers reporting a low incidence of sarcomas with mouse fat used much less vigorous methods of extraction. When mouse fat prepared by

a method similar to that described by Morton and Mider (5) was used as a solvent, the tumor incidence was only 15 per cent at 9 months, as compared to 85 per cent for the corn oil group (Table II). The addition of 0.1 mgm. of α-tocopherol to this mouse fat had no influence on the final incidence of sarcomas. The food intake was controlled for the mice in the experiment recorded in Table II, since various investigators have shown that tumor formation can be retarded in mice by caloric restriction (12, 13). To make certain that differences in caloric intake were not responsible for the comparatively low incidence of tumors in mice injected with mouse fat, all the groups in this experiment were given an amount of food equivalent to 9.7 calories per mouse per day (12). Since these mice consumed all their daily allotment of food, the difference in tumor incidence is not readily explained on a dietary basis.

The effect of fatty acid esters and of α-tocopherol on sarcogenesis.—Fats and oils obtained from animal or vegetable sources are relatively crude substances of variable composition, which differ considerably both as to the degree of saturation and the size of the fatty acid molecules. To obtain data relative to the effect of chain length and unsaturation of the fatty acids on tumor formation, several purified fatty acid esters were used. The effect of α-tocopherol on tumor formation with these esters was also tested.

The result of the first experiment of this type is shown in Table III. An unexpected finding was the low incidence of neoplasms in the groups injected with benzpyrene dissolved in methyl caprate; only 3 per cent of the mice that received 0.1 mgm. of the hydrocarbon in this ester had palpable tumors by 9 months. The addition of 0.2 mgm. of α-tocopherol increased the number of tumors somewhat, but unfortunately a considerable number of mice from this group died early in the experiment so that the apparent increase in number of tumors must be accepted with caution.

The incidence of tumors with ethyl palmitate or ethyl stearate was considerably above that observed with the shorter chained methyl caprate, and α-tocopherol had little or no influence on the final outcome (Table III). Tumor incidence varied from 33 per cent with ethyl stearate to 42 per cent with ethyl palmitate. The effect of ethyl stearate was retested in a second

TABLE II: THE EFFECT OF MOUSE FAT AND CORN OIL ON SARCOGENESIS UNDER CONDITIONS OF EQUAL FOOD INTAKE

(Each mouse received a single subcutaneous injection of 0.1 cc. of oil containing 0.1 mgm. of 3,4-benzpyrene. ABC albino mice of both sexes were used. The mice received 9.7 cal./mouse/day [12].)

Lipid vehicle	α-Tocopherol (0.1 mgm. in each 0.1 cc. of lipid)	Number of mice (effectual total)	Cumulative tumor count: After injection of benzpyrene, months 4	5	6	7	8	9	Number of mice tumor-free and alive at end of experiment	Tumors based on effectual total, per cent
Mouse fat *	0	40	0	0	2	4	4	6	34	15
	+	40	2	2	3	3	3	5	35	13
Corn oil **	0	39	13	23	26	31	32	33	6	85

* Prepared by extracting fresh mouse carcasses with Skelly solve B.
** Mazola.

TABLE III: THE EFFECT OF FATTY ACID ESTERS WITH AND WITHOUT TOCOPHEROL ON SARCOGENESIS

(Each mouse received a single subcutaneous injection of 0.1 cc. of the vehicle containing 0.1 mgm. of 3,4-benzpyrene. ABC albino mice of both sexes were used.)

Lipid vehicle	α-Tocopherol (0.2 mgm. in each 0.1 cc. of lipid)	Number of mice (effectual total)	Cumulative tumor count: After injection of benzpyrene, months 3	4	5	6	7	8	9	Number of mice tumor-free and alive at end of experiment	Tumors based on effectual total, per cent
Methyl caprate	0	33	0	0	0	0	0	1	1	30	3
	+	18	0	0	0	1	1	2	3	14	17
Ethyl palmitate	0	31	0	4	8	8	11	12	13	17	42
	+	28	0	1	5	7	9	10	10	17	36
Ethyl stearate	0	27	0	3	4	6	6	8	9	16	33
	+	32	0	3	5	5	5	6	7	21	22
Ethyl oleate	0	24	1	4	8	13	14	16	17	7	71
	+	26	1	9	13	15	16	16	16	6	62
Ethyl linolate	0	32	0	0	1	1	2	3	6	25	19
	+	16	0	3	5	6	8	9	9	7	56

experiment, where each mouse received an injection of 0.1 cc. of tricaprylin in which 250 mgm. of the ester plus 0.1 mgm. of benzpyrene had been dissolved. After 9 months 74 per cent of the mice had tumors, essentially the same result as observed in the control group (Table IV).

The outcome with ethyl oleate varied somewhat among the experiments. In the first run 71 per cent of the mice had palpable sarcomas at 9 months (Table III), as compared to 45 per cent in another experiment The final incidence was higher, also, in the mice getting the tocopherol plus the ethyl oleate; 77 per cent at 9 months as compared to 45 per cent (Table V), but this result was not observed in a previous experiment (Table III). The results recorded in Table V are probably the more significant of the 2 experiments, since the number of animals alive at 3 months was greater in the latter series. A large number of the mice listed in Table III died early in the experiment as the result of a concurrent infection.

TABLE IV: THE EFFECT OF FATTY ACID ESTERS AND LECITHIN ON SARCOGENESIS

(Each mouse received a single subcutaneous injection of 0.1 cc. containing 0.1 mgm. of 3,4-benzpyrene. Strain C mice of both sexes were used.)

Lipid vehicle	Number of mice (effectual total)	Cumulative tumor count: After injection of benzpyrene, months 4	5	6	7	8	9	Number of mice tumor-free and alive at end of experiment	Tumors based on effectual total, per cent
Tricaprylin (0.1 cc.)	37	2	12	21	23	23	25	11	68
Ethyl stearate (250 mgm. in 0.1 cc. tricaprylin)	39	2	13	19	24	27	29	9	74
Ethyl oleate (250 mgm. in 0.1 cc. tricaprylin)	38	2	10	13	17	23	23	15	60
Ethyl linolate (250 mgm. in 0.1 cc. tricaprylin)	38	0	2	6	8	10	11	24	29
Lecithin (100 mgm. in 0.1 cc. tricaprylin)	39	1	7	11	12	13	15	19	38

TABLE V: THE EFFECT OF CERTAIN LIPID SOLVENTS ON SARCOGENESIS WITH 3,4-BENZPYRENE

(Each mouse received a single subcutaneous injection of 0.1 cc. of the lipid containing 0.106 mgm. of 3,4-benzpyrene. ABC mice of both sexes were used.)

Lipid vehicle	α-Tocopherol (0.2 mgm. in each 0.1 cc. of lipid)	Number of mice (effectual total)	Cumulative tumor count: After injection of benzpyrene, months 3	4	5	6	7	8	9	Number of mice tumor-free and alive at end of experiment	Tumors based on effectual total, per cent
Ethyl linolate	0	40	1	1	1	1	1	1	1	36	3
	+	35	1	1	5	8	9	9	10	22	29
Ethyl oleate	0	40	1	5	12	16	17	18	18	18	45
	+	43	1	10	26	30	31	33	33	8	77
Lard filtrate	0	41	0	5	13	19	23	25	25	16	61
Lard residue	0	41	0	0	2	3	3	3	3	21	7

performed in an identical manner (Table V). Ethyl oleate dissolved in tricaprylin exerted no appreciable influence on the development of sarcomas (Table IV). The addition of 0.2 mgm. of α-tocopherol to 0.1 cc. of ethyl oleate accelerated the formation of tumors in both experiments in which it was tested. Thus at 5 months 50 per cent of the mice listed in Table III and 60 per cent of those in Table V that had been injected with ethyl oleate containing tocopherol had sarcomas, as compared to an incidence of 33 per cent and 30 per cent respectively in the groups injected with the same ester but in the absence of any antioxidant.

When ethyl linolate was used as a solvent the rate of tumor formation was retarded, and the final incidence of tumors decreased. Thus 19 per cent of the mice had sarcomas at 9 months, as compared to 42 per cent in the group injected with ethyl palmitate (Table III); when this experiment was repeated the incidence of tumors was only 3 per cent at 9 months (Table V). The addition of ethyl linolate to tricaprylin also decreased the tumor incidence from 68 to 29 per cent (Table IV). When α-tocopherol was added to ethyl linolate, the formation of tumors was accelerated and the final incidence increased. Thus

56 per cent of the mice injected with ethyl linolate containing the antioxidant had sarcomas at 9 months, as compared to a 19 per cent incidence in the controls (Table III). Similar results were obtained in the group shown in Table V, where the final incidence of neoplasms was 29 per cent and 3 per cent respectively.

The effect of lecithin and of lard fractions on sarcogenesis.—Since fatty acids compose the largest part of the lecithin molecule, the effect of this phospholipid on sarcogenesis was investigated. Mice were given a single subcutaneous injection of 0.1 cc. tricaprylin containing 100 mgm. of lecithin, and a retardation was observed on the rate of tumor formation and on the final incidence. At 9 months only 38 per cent of the mice that received lecithin in the injections had tumors, as compared to 68 per cent for the controls (Table IV). These results are in agreement with those recently published by Weil-Malherbe and Dickens (15).

Leiter and Shear (3) observed considerable variation in the rate of tumor formation when different fractions of leaf lard were used as the solvent for benzpyrene. Many more neoplasms were formed when the portion of lard that is liquid at 38° C. was employed as the vehicle for the carcinogen than when the lard residue was used in the same manner. The results obtained in the present experiments (Table V) are in excellent agreement with those reported by Leiter and Shear; 61 per cent of the mice had sarcomas at 9 months when lard filtrate was used as the solvent, as compared to an incidence of 7 per cent with the lard residue fraction.

The rate of disappearance of 3,4-benzpyrene in vivo *and* in vitro *in solvents of varying degrees of autoxidizability.*—Previous investigations from this laboratory have demonstrated that benzpyrene undergoes a coupled oxidation when present in solution with an autoxidizing lipid, and that the rate of hydrocarbon destruction parallels the degree of fat oxidation (6, 7). In the present investigation, samples of the carcinogen-lipid mixtures listed in Table V were placed in test tubes, kept in the dark, and analyzed for the content of benzpyrene at regular intervals. Four groups of 25 mice each were injected as outlined in Table V, and 5 from each group were sacrificed at the same intervals and the amount of benzpyrene in the subcutaneous tissues was determined.

Benzpyrene disappeared much more rapidly from the vehicle in the subcutaneous tissues than from the same solvent in the test tube, but there was no obvious relationship between the rate of disappearance of hydrocarbon from the tissues either with the degree of autoxidizability of the lipid or with the rate of tumor formation (Tables V and VI). In general, the rate of disappearance of hydrocarbon from the tissues was comparable in all the groups at any one period, although a considerable variation existed among the individual mice of any one group. By the end of the first week from 50 to 77 per cent of the carcinogen was no longer detectable, at the end of the fourth week 81 to 99 per cent had been destroyed, and after 8 weeks essentially all was gone.

The *in vitro* studies on the destruction of benzpyrene in autoxidizing lipids confirmed our previous findings (6, 7). The disappearance of the hydrocarbon was faster when dissolved in ethyl linolate than when mixed with ethyl oleate, and the incidence of tumors was greater with the latter ester. Tocopherol retarded hydrocarbon destruction and accelerated sarcogenesis with both lipids. Thus when tocopherol was added to ethyl linolate only 5 per cent had disappeared by 2 weeks, as compared to 17 per cent when no antioxidant was used (Table VI). However, by 8 weeks the same amount of benzpyrene had disap-

TABLE VI: THE RATE OF DESTRUCTION OF 3,4-BENZPYRENE IN VARIOUS SOLVENTS *in Vitro* AND IN THE SUBCUTANEOUS TISSUES OF MICE

(Mice treated as in Table V were used for these determinations. Analyses were made on 0.1 cc. of the mixture set aside in test tubes for varying periods and on tissues removed from a wide area surrounding the subcutaneous injections. Five mice from each group were analyzed at each period.)

		Amount of 3,4-benzpyrene present in samples at intervals																				
		Start			1 week				2 weeks				4 weeks				8 weeks					
		in vitro	*in vivo*		*in vitro*	*in vivo*			*in vitro*	*in vivo*			*in vitro*	*in vivo*			*in vitro*	*in vivo*				
Lipid vehicle	α-Tocopherol (200 μgm./cc.)	μgm./0.1 cc.	μgm./mouse	% recovered	% destroyed	μgm./mouse Av.	μgm./mouse Range	% destroyed	% destroyed	μgm./mouse Av.	μgm./mouse Range	% destroyed	% destroyed	μgm./mouse Av.	μgm./mouse Range	% destroyed	% destroyed	μgm./mouse Av.	μgm./mouse Range	% destroyed		
Ethyl linolate	0	106	101	95	7.0	30	10-47	71.7	17.0	28	19-45	72.6	46.0	15	0.5-44	85.7	72.0	0.3	0.2-0.5	99+		
	+	106	99	93	0.0	31	14-45	70.8	5.1	33	8-57	68.0	37.4	20	11 -41	81.1	72.4	1.7	0.5-4	99		
Ethyl oleate	0	106	100	94	0.0	24	18-33	77.4	7.5	11	3-29	89.6	11.0	1.2	0 -4	99	15.5	0.2	0.1-0.3	99+		
	+	106	98	92	0.0	30	5-60	71.7	3.4	18	8-29	83.0	7.7	8	1 -12	92.5	10.0	0.6	0 -2	99+		
Lard filtrate	0	106	109	103	0.0	39	31-55	63.2	2.8	11	5-19	89.6	5.7	1.5	0.5-3	98.6	6.6	0.3	0 -1	99+		
Lard residue	0	106	97	92	0.0	53	39-68	50.0	2.4	23	1-49	78.3	7.0	8	0 -30	92.5	7.4	0.2	0 -0.5	99+		

peared in both samples regardless of whether tocopherol was present or not. With ethyl oleate as the solvent the same general trend was seen, but the differences were not so pronounced as those with the more unsaturated fatty acid ester. There was also a pronounced difference in tumor incidence in mice injected with benzpyrene either in lard filtrate or residue (Table V), but no obvious correlation could be made with the rate of hydrocarbon disappearance either *in vitro* or *in vivo*.

DISCUSSION

Several suggestions have been offered to explain why some lipoidal solvents enhance sarcogenesis while others retard it. Some investigators have postulated that some positive substance is present in oils that is responsible for the effects. Thus from the results of the present experiments or from the work of Weil-Malherbe and Dickens (15) one might assume that lecithin contains an agent that retards tumor formation. Similarly the study of Leiter and Shear (3) could be taken to indicate that a retarding influence is present in glycerides that contain saturated fatty acids of high molecular weight; *i.e.*, tristearin and tripalmitin. Factors inhibitory to the genesis of tumors have also been postulated as occuring in other fatty substances, but such claims have lacked confirmation (15).

Some investigators have surmised that differences in rate of removal of various solvents might account for the differences in sarcogenesis with the various lipids. Peacock and Beck (9) attributed the anticarcinogenic action of homologous fat to its more rapid elimination from the injection site, with a correspondingly rapid removal of the carcinogen. However, the observations of Dickens and Weil-Malherbe (1) and our own findings do not support this theory. In the present experiment a considerable difference in tumor incidence was observed depending on whether the solvent employed was the ethyl ester of linoleic or oleic acid (with or without tocopherol), lard filtrate, or lard residue, yet the disappearance of benzpyrene from the subcutaneous tissue in all these groups was essentially the same. Weil-Malherbe and Dickens (15) demonstrated that sarcomas appeared earlier with cod liver oil than with tricaprylin as solvents for benzpyrene, but the hydrocarbon on gross examination disappeared more rapidly from the site of injection with the former solvent than with the latter. Additional work certainly is indicated on this aspect of the problem.

Since various fats differ considerably in their degree of autoxidizability, this factor must also be considered when searching for an explanation of the variations in tumor formation with different solvents. Benzpyrene and methylcholanthrene have been shown to be destroyed rather rapidly when dissolved in unsaturated fats, and the rate of disappearance has been correlated with the amount of autoxidation exhibited by the particular lipid (6, 7). Most vegetable fats contain a considerable amount of antioxidants (8) and in these lipids the hydrocarbon remained relatively intact (7), whereas the content of inhibitols in lipids (lard) from animals is low (8) and the rate of destruction of the carcinogen in such fats was correspondingly rapid (7). However, the variation in carcinogenicity of benzpyrene in different oils can not be explained from such studies. In the present study there was a correlation between the number of sarcomas formed and the stability of the lipid *in vitro* with some fats, but this did not prove to be the case with all oils, and no relationship seemed to exist when the rate of tumor formation was compared to the disappearance of the carcinogen from the tissues. However, the methods employed in the present experiment were not sufficiently sensitive to detect the minute amounts of carcinogen actually required for the transformation of a single cell into a neoplastic one. Were such technic available, an effect of antioxidants on the level of hydrocarbon might be demonstrated.

It is probable that no one theory will suffice to explain the differences in tumor formation when various lipids are employed as solvents. Other factors such as differences in the exact site of injection, the amount of dispersal of the injected solution, or differences in tissue response such as the amount of irritation, variation in the degree of encapsulation, vascularization, or phagocytic infiltration all must be considered in an investigation of this type (15).

SUMMARY

The effect of various lipids on sarcogenesis when used as solvents for 3,4-benzpyrene was compared with certain chemical and physical characteristics of the lipids. The vehicles employed were ethyl stearate, ethyl oleate, ethyl linolate, ethyl palmitate, methyl caprate, tricaprylin, lecithin, mouse fat, corn oil, cottonseed oil (largely freed of antioxidants), "lard filtrate," and "lard residue." A pronounced retardation of tumor formation was observed with methyl caprate, ethyl linolate, lecithin, lard residue, or a petroleum ether extract of mouse carcasses.

With some lipids there was an indication that their effect on sarcogenesis could be correlated with their susceptibility to autoxidation *in vitro* and with the corresponding destruction of the dissolved hydrocarbon. Thus the addition of α-tocopherol to ethyl linolate, ethyl oleate, or cottonseed oil (largely freed of antioxidants) decreased the rate of destruction of 3,4-benzpyrene and favored the formation of tumors.

However, tocopherol also enhanced the genesis of sarcomas when added to the saturated fatty acid ester, methyl caprate, a substance that is not susceptible to autoxidation. Furthermore, there was relatively little destruction of benzpyrene *in vitro* when it was dissolved in lard residue, yet this solvent had a striking inhibitory influence on the development of tumors.

No relationship was observed when the rate of disappearance of hydrocarbon from the tissues was compared to the incidence of tumor formation. Thus when 3,4-benzpyrene was dissolved in ethyl linolate or ethyl oleate (each with and without added tocopherol), lard residue, or lard filtrate and the mixtures were injected subcutaneously, the carcinogen disappeared at the same rate from the tissues of all the groups, but the final incidence of sarcomas varied from 3 to 77 per cent depending on the solvent used.

It seems evident from the results of these experiments that the variation in tumor formation observed when different lipids are used as solvents for a carcinogen cannot be attributed to any single characteristic of the oils. Instead, it appears that a variety of factors concerning the chemical and physical characteristics of the lipid, and the differences in tissue response to the solvent, all play an important role in carcinogenesis.

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Influence of Age on Calcium in Epidermal Carcinogenesis Induced by Methylcholanthrene in Mice*

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The major research project of Barnard Hospital is to integrate the changes that occur in experimental epidermal methylcholanthrene carcinogenesis in mice. A summarizing report of progress has recently been published (4). It is important to determine the influence of age on the kind of carcinogenesis. We have already found that cutaneous applications of 0.6 per cent methylcholanthrene produce cancers more quickly and in a higher percentage of young than of old New Buffalo mice, whereas the response of young and old CBA mice (a strain more resistant to this carcinogen) is approximately the same (3). It therefore appears that hereditary constitution can submerge the age influence. Since other experiments in the major project have demonstrated significant decreases in epidermal calcium and iron in New Buffalo mice treated with methylcholanthrene (1, 2, 6) the following experiments were made to ascertain the correlation, if any, between this difference in the response of young and old mice of these two strains and changes in epidermal calcium.

EXPERIMENTAL PROCEDURE

New Buffalo and CBA strains of mice of both sexes were used. For each strain there were two age groups: one 3 to 4 months old and the other 12 to 13 months old. The method of application of carcinogen already has been described (1). It has been somewhat modified from that used in our first experiments (3) in order to provide sufficient epidermis for analysis. The calcium content of completely ashed (at 450° C in a muffle furnace) samples was determined by the method (on a micro scale) of Lindner and Kirk (5). Previously we used nucleoprotein phosphorus as a basis of reference for the amount of tissue, but in this paper the results are expressed in mgm. of calcium per 100 mgm. of epidermis (wet weight). This change was made because experiments clearly demonstrated that quantitative alterations in calcium and other minerals were the same, whether wet weight or nucleoprotein phosphorus was employed as the basis of reference, and wet weight is simpler.

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RESULTS

Inspection of Table I shows that the epidermal calcium content of the old New Buffalo mice is about 18 per cent greater than that of young mice of the same strain. After 3 applications of the carcinogen the decrease in the calcium content of about 58 per cent was the same for both age groups. Furthermore, despite repeated applications for a period of 2 months, the diminution of calcium was maintained at about the same level for both groups.

The changes in the calcium content of epidermis from young and old CBA mice, brought about by similar treatment with methylcholanthrene, are shown in Table II. The old group had about 27 per cent more calcium than the young group to start with. The diminution in calcium content induced by 3 applications of the carcinogen in the young CBA was about 30 per cent, while the drop in the old group was some 44 per cent. Repeated treatments of the epidermis of the old mice for 6 weeks, 1 month, and 2 months produced a decrease in the calcium content by 35 per cent, 44 per cent, and 36 per cent respectively, while in the young group under identical conditions the drop in calcium amounted to 20 per cent, 20 per cent, and 18 per cent respectively, or about 50 per cent less than that of the old group. Treatment of the young CBA mice for 3 and 4 months did not cause a further drop in the calcium content.

The results of all the analyses are shown graphically in Fig. 1, in which the percentage of decrease in calcium is plotted against the time. Upon treatment of the epidermis of both age groups of the New Buffalo strain with the carcinogen, the diminution of the calcium was pronounced, and remained essentially the same up to 60 days, at which time the mice had received 24 applications. On the other hand the drop in the calcium content for both age groups of the CBA strain was distinctly less after 3 treatments with the carcinogen, a greater decrease occurring in the old group. The initial diminution produced by 3 treat-

TABLE I: CALCIUM CONTENT OF YOUNG AND OLD NEW BUFFALO MICE

Young mice (3-4 months old *)				Old mice (12-13 months old)			
No. of mice	No. of paintings	Time after first painting to killing of mice, days	Ca per 100 mgm. tissue, mgm.	No. of mice	No. of paintings	Time after first painting to killing of mice, days	Ca per 100 mgm. tissue, mgm.
			NORMAL, UNTREATED MICE				
7			0.035	10			0.044
8			0.042	8			0.047
11			0.050	10			0.066
11			0.040	10			0.057
10			0.040	9			0.061
12			0.052	8			0.050
—				—			
59 (total)			Average 0.043	55 (total)			Average 0.054
			METHYLCHOLANTHRENE-TREATED MICE				
7	3	10	0.019	6	3	10	0.022
4	3	10	0.019	6	3	10	0.023
5	3	10	0.017	7	3	10	0.023
4	3	10	0.019	—			
—				19 (total)			Average 0.023
20 (total)			Average 0.019				
5	6	20	0.016	5	6	20	0.020
5	6	20	0.011	5	6	20	0.023
7	6	20	0.023	—			
7	6	20	0.013	10 (total)			Average 0.022
—							
24 (total)			Average 0.016				
5	12	30	0.016	10	12	30	0.018
5	12	30	0.012	11	12	30	0.018
7	12	30	0.019	—			
7	12	30	0.014	21 (total)			Average 0.018
—							
24 (total)			Average 0.015				
5	24	60	0.022	8	24	60	0.025
6	24	60	0.021	7	24	60	0.019
5	24	60	0.023	—			
—				15 (total)			Average 0.022
16 (total)			Average 0.022				

* From previous publication (5).

TABLE II: CALCIUM CONTENT OF YOUNG AND OLD CBA MICE

Young mice (3-4 months old*)				Old mice (12-13 months old)			
No. of mice	No. of paintings	Time after first painting to killing of mice, days	Ca per 100 mgm. tissue, mgm.	No. of mice	No. of paintings	Time after first painting to killing of mice, days	Ca per 100 mgm. tissue, mgm.
			NORMAL, UNTREATED MICE				
10			0.045	10			0.054
8			0.046	8			0.052
11			0.036	9			0.054
13			0.035	8			0.059
12			0.039	—			—
12			0.037	35 (total)			Average 0.055
—			—				
66 (total)			Average 0.040				
			METHYLCHOLANTHRENE-TREATED MICE				
7	3	10	0.034	6	3	10	0.035
8	3	10	0.029	5	3	10	0.033
7	3	10	0.033	5	3	10	0.026
8	3	10	0.034	—			—
10	3	10	0.024	16 (total)			Average 0.031
10	3	10	0.021				
8	3	10	0.022				
—			—				
58 (total)			Average 0.028				
7	6	20	0.030	7	6	20	0.039
9	6	20	0.036	7	6	20	0.033
8	6	20	0.033	—			—
7	6	20	0.029	14 (total)			Average 0.036
—			—				
31 (total)			Average 0.032				
8	12	30	0.034	7	12	30	0.028
8	12	30	0.031	8	12	30	0.035
—			—	—			—
16 (total)			Average 0.032	15 (total)			Average 0.032
6	24	60	0.029	6	24	60	0.032
7	24	60	0.031	6	24	60	0.038
8	24	60	0.036	—			—
—			—	12 (total)			Average 0.035
21 (total)			Average 0.033				
8	36	90	0.031				
9	36	90	0.030				
—			—				
17 (total)			Average 0.030				
17	48	120	0.030				

ments in both age groups was not further accentuated by painting the epidermis at regular intervals for 2 months.

These determinations of the epidermal calcium content of young and old mice of both strains do not afford an explanation of the observation described in a previous paper (3), that young New Buffalo mice

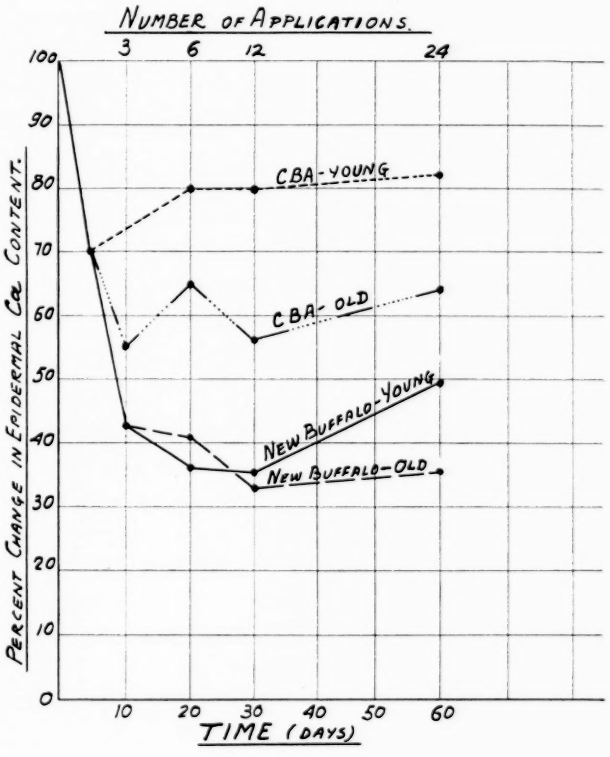

FIG. 1

develop tumors earlier in a higher percentage of individuals than do the old ones, for there is no significant difference in decrease in the calcium content between the young and old groups. But there is some correlation between tumor development and depression in epidermal calcium in the CBA strain, since the older mice develop tumors somewhat earlier and in a higher percentage than do the young, while at the same time there is a greater fall in the calcium content in the older than in the younger group.

Microscopical changes of the epidermis of both strains treated with the carcinogen were more or less similar, except that after 2 months the epidermis of the old CBA mice was much less hyperplastic than that of the old New Buffalo group. It is not only probable, but quite certain, that factors other than the calcium content operate in epidermal carcinogenesis and may explain the differences in response to carcinogen, not only between the young and old mice of each strain, but also between both strains.

SUMMARY

The role of calcium as a factor in the age difference to the response of epidermis of old and young mice of the New Buffalo and CBA strains of mice to methylcholanthrene has been investigated.

Old mice of both strains were found to contain more epidermal calcium than the young. The epidermis of both age groups of the New Buffalo strain responded similarly when treated with methylcholanthrene; that is, they underwent a nearly 50 per cent decrease in the calcium content. However, the diminution in the calcium content of the CBA strain was less than that of the New Buffalo, and the young group showed about 50 per cent less drop than did the old.

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Chronic Inflammation and Carcinogenesis

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INTRODUCTION

In discussing the etiology of carcinoma of the mouse mamma Bittner (5) wrote that three causes have been recognized, but that still others may be involved. Those already identified are an inherited susceptibility, adequate hormonal stimulation, and the milk influence.

Is chronic irritation a fourth?

It will be recalled that Borrel (8) found nematodes in young mammary carcinomas and their surroundings, and suggested that the parasites might serve as carriers of a cancer virus. Similar worms were described in the subcutaneous tissues of old male and female mice by Haaland (16), who gave a minute description of the inflammatory and sclerotic changes that accompanied their presence. In the female these lesions were associated with nodular hypertrophy of the epithelium and even with cancer, and Haaland suggested that the epithelial hypertrophy and the tumors arising on this basis could be brought into relation with the inflammatory changes. As infestation with the nematodes was more commonly found than neoplasia it was proposed, also, that if such a relation actually existed it was a mediate one, and analogous with that of syphilis, say, to cancer of the tongue.

But in a mouse colony kept under exceptionally favorable hygienic conditions Dobrovolskaia-Zavadskaia and Kobozieff (13) found nodular hypertrophy in the absence of nematodes, and it is now referred to hormonal rather than parasitic causes. Furthermore, chronic irritation has been deposed from its once proud state as the sole cause of malignant growth, for many tumors are now known to arise in its absence. Nevertheless, it does seem at times to be *a* cause, if not *the* cause.

Its significance in carcinoma of the mouse mamma has been investigated in several different ways.

Dobrovolskaia-Zavadskaia (10, 11) sought to influence the localization of mammary carcinoma in her RIII strain, with an incidence of 71 per cent, by inserting radon tubes subcutaneously in the groin. But although both dosage and time were adequate, carcinomas arose at other sites in 22 of the 47 mice employed. In 11 animals neoplasms did develop about the tubes, to be sure, but almost all were sarcomas. The author concluded that radon is incapable of influencing the localization of mammary carcinoma in the mouse; this, she thought, is determined by some endogenous factor.

It is to be noted, however, that even the smallest amounts of radon elicited considerable necrosis, and it is not impossible that the mammary tissue about the tubes was too badly damaged to give rise to carcinoma.

In a later paper (12) it was said that the injection into a mamma of 0.0025 to 0.1 mgm. of 1,2,5,6-dibenzanthracene did not increase the proportion of carcinomas developing in it. This is a more convincing experiment, for no doubt these small doses were far less destructive than the irradiation.

It is not stated in either of these articles whether the mice were virgin or breeders, but internal evidence suggests the latter.

Bischoff and Long (3) injected a solution of zinc sulfate into the mamma about each nipple of virgin mice belonging to a high tumor strain. Among the 106 animals that appear in the final calculation 73 per cent of the controls, but only 38 per cent of those treated, developed mammary carcinomas. As the injections gave rise to considerable local necrosis the decrease in cancer incidence was attributed by the authors to the destruction or inhibition of cancer-susceptible cells.

The development of thymic tumors in virgin female mice was not influenced by the injection of zinc chloride into the gland (4).

In addition to the exogenous agents just mentioned, endogenous also have been tested. Bagg (1, 2), and later Bogen (7), found that occlusion of the nipples on one side of the body caused more tumors to arise there than on the untreated side, presumably because of the irritation set up by the stagnant and decomposing milk. In the similar experiment of Fekete and Green (14) not only were there more carcinomas on the treated side, but these arose at an earlier age. Stagnation in itself, however, was powerless to elicit tumors in a low-cancer strain.

In all these experiments with endogenous agents

the mice were bred, and in Bagg's, at least, intensively bred. Where exogenous agents were introduced they appear to have been too strong in two cases out of three, and in the third the larger doses of dibenzanthracene might conceivably have caused some damage at least. Hence there still seemed room for an investigation of the effect of an extremely mild irritant in virgin mice of a high cancer strain, where the element of normal or forced breeding would not enter as a possible disturbing factor.

MATERIALS AND METHODS

Three silk threads, black for easy identification later and from 0.5 to 1.0 cm. in length, were sewn into the mamma of mice subjacent to the upper right inguinal nipple, after a flap of skin and mamma had been reflected under ether. Following closure of the wound the 180 animals thus treated were returned to their cages in groups of 6, where they were maintained on a diet of 1 part Purina dog chow crackers and 2 parts Rockland rat diet. Both food and water were offered *ad libitum*.

These mice were from an RIII strain that has been brother-to-sister bred in this laboratory for the past 5 years or so, and almost all were from 6 to 7 weeks old at the time of operation; the negligible remainder were from 8 to 11 weeks old. All were virgin, and remained so throughout the experiment, and excepting those killed for microscopic examination of the tissues about the threads all were allowed to live out their natural span.

After the acute foreign body reaction had subsided the threads excited even less disturbance than had been anticipated, the sole response to their presence having been a thin connective tissue capsule.

With the exclusion of 30 mice that were killed for histological examination; or had lymphatic tumors of mammary cysts; or died before the age of 192 days, when the first mammary carcinoma was found, 150 remain for consideration.

Of these, 115 died without tumors at the following ages:

221-294 days	6
305-399	13
401-496	14
511-597	29
601-697	31
700-780	18
811-868	4
Total	115

Mammary carcinomas, verified by microscopic examination, arose at the following ages in 35 (23 per cent) of the 150 effectual mice; only single, or first, tumors are taken into account:

Days	Mice	Days	Mice	Days	Mice
192	1	412		600	
252		441		615	
258**		451		622	
294**	5	451**	6	628	6
294		453**		678	
295		461*		682	
342		538		783	1
349*		542			—
349		544			Total 35
363		558	7		
369	9	565*			
370		577			
374		577			
375**					
375**					

* Indicates tumor in upper right inguinal mamma, but threads not seen.

** Indicates tumor close to threads.

DISCUSSION

Thus 9 out of the 35 carcinomas were found in contact with, or near, the foreign body introduced. But in order to decide whether this proportion has any significance it is necessary to know the distribution of mammary tumors in the strain of mice employed. Examination of the records showed that in 500 mice examined in order of their running numbers, and therefore without any selection whatsoever, single tumors or those first to arise were evenly divided among the axillary and inguinal mammae, as was to have been expected from the work of other investigators:

Right axilla	20.2%
Left "	22.0
Right groin	22.4
Left "	20.0
Root of tail	0.6
Anterior and posterior cervical regions	14.8

Accordingly, among 35 mammary carcinomas 8 might have been expected to arise in the right inguinal mammary region and 9 were found there. This statement is made in full realization of the hazards involved in calculating 22 per cent of 35. As Oberling (18) has so well said, many investigators ". . . . have the deplorable habit of reckoning percentages on fewer than one hundred animals. If a tumor should regress in 7 out of 10 animals this means 7 out of 10; just that, and not 70 per cent. The outcome might have been entirely different if 100 animals actually had been used."

But as the outcome in this case coincides almost exactly with expectation and is supported, furthermore, by the experience of Dobrovolskaia-Zavadskaia and Adamova (12) with dibenzanthracene, it may perhaps be accepted as probably reliable. The experiment, long and laborious, is reported for what it is worth, since there is little incentive to continue such an investigation when the promised reward is so small.

If the incidence of tumors was not increased, neither was their inception accelerated, as the distribution of asterisks might at first glance suggest. A decline of the cancer incidence with age is known to occur in some strains of mice, including the RIII, and has recently been reported by Heiman (17) for these mice under the conditions obtaining in this laboratory and observed by Twombly (20), also, in virgin females of the same strain.

The incidence in the present experiment was inexplicably and disappointingly low, for it was thought that the number of animals employed was large enough to provide respectable figures, other investigators having reported 52.13 ± 5.15 (15), 70 (19), and 74.7 (20) per cent in virgin females belonging to American branches of this French strain. Spontaneous decrease in the incidence of mammary cancer among mice is a well recognized event, which has been reviewed by Bittner (6) and of which Burrows (9) shortly afterward supplied another example. Certainly it is a far more credible explanation of the low incidence in this experiment than an assumption that the fall was caused by the presence of silk threads.

SUMMARY

Compared with the three causes so far known, the constant presence of a mild irritant in the mamma of RIII virgin female mice was of no importance in determining the site or accelerating the initiation of mammary carcinoma.

ACKNOWLEDGMENT

I am indebted to Mr. Anton Samuel for his expert care and supervision of the animals.

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Influence of Nontoxic Amounts of Estradiol upon Carcinogenesis in Intact and Castrated Male and Female Mice of the Marsh-Buffalo Strain

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Experimental work (1, 2) on the influence of exogenous estrogens upon the development of tumors in the Marsh-Buffalo strain indicates that nontoxic doses of estrogens are without influence upon the development of tumors of the mammary gland in intact females and do not produce these tumors in intact males. Toxic doses enhance the development of such neoplasms in females; these toxic doses do not produce mammary tumors in intact males, but a low incidence results in castrated males. In general an increase in lymphoid tumor formation has accompanied the administration of toxic doses of estrogens in both the intact and castrated female. The intact male, however, has been resistant to lymphoid tumor formation, though not the castrated male.

It is not known whether tumor formation can be induced in Marsh-Buffalo castrated mice of both sexes by nontoxic doses of estrogens. In view of the negative response of intact male and female mice to such doses this information would be desirable, and is the subject of the present report.

EXPERIMENTAL

Female mice.—For the purpose of compilation, 40 trios of litter mates were used. There were originally 9 mice in addition to the 40 trios used for compilation. Of these, at the second month of age, 1 with curved long teeth and 2 with abnormalities of the vagina, and their respective litter mates were removed from the experiment.

Castration was performed at the age of 30 days. The castrated mice and the treated intact mice received a total of 0.98 mgm. of estradiol per mouse in a 7 month period, beginning at the age of 6 weeks. The controls received 0.98 cc. of sesame oil during this period, this being the amount of sesame oil in which the 0.98 mgm. of estradiol was dispersed. The estradiol was administered once a week, subcutaneously or intramuscularly, at a site distant from the mammary area.

One control, 2 treated intact, and 1 castrated mouse were removed from the experiment before the age of 15 months because of death not related to tumor formation. The incidence of this figure is so low and so evenly distributed that it would not influence tumor incidence compilation and may be ignored. From the 15th to the 18th month of age no controls, 3 treated intact mice, and 5 castrated mice, died of causes not related to tumor formation.

Body weight was recorded at 6 week intervals. At the seventh and ninth months of age the weight of the treated intact mice was 4 per cent below that of the controls. This difference is less than twice the standard deviation of the mean. At other periods there was no difference for these 2 groups. From the 11th month on, the castrated mice weighed approximately 11 per cent more than the controls.

The 40 per cent cumulative incidence of adenocarcinoma at the 16th month of age in the control mice is somewhat lower than the incidence originally reported by Marsh for this strain of mice and confirmed by data from our laboratory. This decrease in incidence must be attributed to the sesame oil, an observation that confirms similar data previously reported.

On the basis of cumulative per cent incidence, there is never a significant difference between controls and intact treated mice in the development of adenocarcinoma. For the castrated treated mice, however, the incidence of 23 per cent at the 18th month of age is significantly less than the 48 per cent observed for the controls. At the 12th month the cumulative incidence of lymphoid tumors is significantly increased for the treated intact mice. The magnitude of the incidence, 13 per cent, is, however, not striking. In the castrated treated mice a significant increase in lymphoid tumors is not apparent.

Deductions based upon cumulative incidence may be deceptive if other causes that remove animals from the experiment are not considered (1). This factor may be corrected for by calculating incidence on the basis of surviving mice, but in the present experiments it does not appreciably affect the deduction made, because the distribution is uniform.

Male mice.—The male mice described in Table II as treated castrated control were litter mates of the females. Thirty-five served as controls and 35 were castrated at the age of 30 days. The castrated males received the same dosage of estradiol on the same schedule as did the female mice. The controls received sesame oil. In addition, 31 male mice were castrated at 30 days of age and served as a control for the influence of castration *per se* upon the development of lymphoid tumors.

velopment of lymphoid tumors. In contrast, the incidence of lymphoid tumors is significantly increased in the treated castrated mice when a comparison is made with the intact and castrated mice that did not receive estradiol. The difference is 2.1 to 2.4 times the standard deviation of the mean from the 15th through the 17th month of age.

At the 16th month of age 1 castrated treated mouse developed a mammary adenocarcinoma. One castrated control developed a fibroma at the 13th month of age.

TABLE I: CUMULATIVE INCIDENCE OF LYMPHOID TUMORS AND OF MAMMARY ADENOCARCINOMA IN INTACT AND CASTRATED FEMALE MARSH-BUFFALO MICE THAT HAD RECEIVED ESTRADIOL, AND IN CONTROLS

Age, months	Treated castrated (40 mice)		Treated intact (40 mice)		Control (40 mice)	
	Lymphoid tumors, per cent	Adeno-carcinoma, per cent	Lymphoid tumors, per cent	Adeno-carcinoma, per cent	Lymphoid tumors, per cent	Adeno-carcinoma, per cent
6	3 ± 2	0	3 ± 2	0	0	0
7	5 ± 3	0	3 ± 2	3 ± 2	0	0
8	5 ± 3	3 ± 2	3 ± 2	3 ± 2	0	0
9	5 ± 3	5 ± 3	8 ± 4	10 ± 5	0	5 ± 3
10	5 ± 3	10 ± 5	10 ± 5	15 ± 6	0	5 ± 3
11	8 ± 4	13 ± 5	10 ± 5	18 ± 6	0	10 ± 5
12	8 ± 4	15 ± 6	13 ± 5	20 ± 6	0	15 ± 6
13	8 ± 4	20 ± 6	18 ± 6	28 ± 7	3 ± 3	20 ± 6
14	8 ± 4	20 ± 6	18 ± 6	28 ± 7	10 ± 5	30 ± 7
15	8 ± 4	20 ± 6	18 ± 6	33 ± 7	10 ± 5	35 ± 7
16	13 ± 5	20 ± 6	20 ± 6	38 ± 7	10 ± 5	40 ± 8
17	15 ± 6	23 ± 7	20 ± 6	40 ± 8	10 ± 5	45 ± 8
18	20 ± 6	23 ± 7	25 ± 7	40 ± 8	15 ± 6	48 ± 8

TABLE II: CUMULATIVE INCIDENCE OF LYMPHOID TUMORS AND DEATH DUE TO CAUSES NOT RELATED TO TUMOR FORMATION IN CASTRATED MALE MARSH-BUFFALO MICE THAT HAD RECEIVED ESTRADIOL, AND IN INTACT CONTROLS

Age, months	Treated castrated (35 mice)		Control (34 mice)		Control castrated (31 mice)	
	Dead of other causes, per cent	Lymphoid tumors, per cent	Dead of other causes, per cent	Lymphoid tumors, per cent	Dead of other causes, per cent	Lymphoid tumors, per cent
9	0	0	9 ± 5	3 ± 3	3 ± 3	0
10	0	3 ± 3	9 ± 5	3 ± 3	6 ± 4	0
11	0	6 ± 4	9 ± 5	3 ± 3	6 ± 4	0
12	3 ± 3	12 ± 5	12 ± 5	3 ± 3	6 ± 4	3 ± 3
13	3 ± 3	15 ± 6	18 ± 6	6 ± 4	10 ± 5	3 ± 3
14	6 ± 4	18 ± 6	18 ± 6	6 ± 4	13 ± 6	6 ± 4
15	9 ± 5	23 ± 7	18 ± 6	6 ± 4	16 ± 6	6 ± 4
16	18 ± 6	29 ± 8	18 ± 6	9 ± 5	23 ± 8	6 ± 4
17	23 ± 7	32 ± 8	30 ± 8	9 ± 5	30 ± 8	10 ± 5
18	23 ± 7	32 ± 8	33 ± 8	9 ± 5	30 ± 8	13 ± 6

At the age of 13 months 18 per cent of the control, 3 per cent of the castrated treated, and 10 per cent of the castrated control mice had been removed from the experiment by death not related to tumor formation. At the 18th month of age these figures were respectively 33, 30, and 23 per cent. At the age of 18 months 9 per cent of the controls, 32 per cent of the treated castrated mice, and 13 per cent of the castrated controls had developed lymphoid tumors.

In the control intact and control castrated males there is no difference in the cause of death from conditions other than lymphoid tumors, or in the development of lymphoid tumors. In contrast, the incidence of lymphoid tumors is significantly increased in the treated castrated mice when a comparison is made with the intact and castrated mice that did not receive estradiol. The difference is 2.1 to 2.4 times the standard deviation of the mean from the 15th through the 17th month of age.

The body weights of the 3 experimental groups did not differ significantly during the course of the experiment.

DISCUSSION

The difference in the effect of estradiol upon castrated male and castrated female mice is rather striking. Since the males and females were litter mates, and the treatment of the respective groups was identical in all respects, a direct comparison of the results is justified. The difference in incidence of mammary adenocarcinoma is 23 per cent for the females versus

3 per cent for the males. This difference of incidence is obviously significant, since it is 3 times the standard deviation of the mean. In experiments previously recorded (1), in which 3.5 times the amount of estradiol was administered, the incidence of mammary tumors in the females was increased over the 23 per cent obtained in the present experiment, if the compilation is made on the basis of surviving mice. In the high dosage series the incidence of mammary tumors in castrated treated male mice was 6 per cent. It is apparent that even at the early age of 30 days factors have come into play in the females predisposing them to the precancerous condition, factors that are almost negligible in the male.

It will be recalled that following the injection of estrins, males from other high tumor strains of mice developed tumors of the mammary gland as frequently as did females, that had been bred (4, 5, 7, 8). Males from low tumor strains developed tumors with greater frequency than did breeding females of the same strain. Neoplasms of the mammary gland have not resulted following estrin treatment in male mice from some of the very low strains, even though prolonged and drastic attempts have been made to produce them (3, 5, 9). Thus the resistance to the action of estrins of the Marsh-Buffalo strain in comparison with other high cancer strains, as previously recorded (1), is further substantiated. No intact male has as yet developed a mammary tumor following the administration of estrin. The 3 tumors that developed in castrated males, 2 on high dosage, 1 on low dosage, indicate that the protection offered by the androgens of testicular origin is of negligible importance, although a slight influence cannot be denied. It should be noted that these tumors of the mammary gland developed 15 or more months after castration, and 7 or more months after the administration of the last dose of estrin. The phenomenon recalls the latent period that characterizes the action of the carcinogenic hydrocarbons.

In the present experiment nontoxic doses of estradiol increased the incidence of lymphoid tumors in the castrated male, as did toxic doses in previous experiments. Moreover, the effect upon lymphoid tumor formation was greater at each dosage level than that producing carcinoma of the mammary gland. The intact Marsh-Buffalo male has been resistant to lymphoid tumor formation through the action of the estrogens, although the normal incidence of lymphoid tumors is the same in males and females. The results of castration bear out in general the observation of Gardner (6), that the male steroid hormone is inhibiting to the action of the estrogens.

CONCLUSION

In female mice of the Marsh-Buffalo strain the maximum amount of estradiol that could be administered without producing toxic symptoms or significant losses in body weight was without effect on the development of mammary tumors in intact mice, and without appreciable effect on the development of lymphoid tumors in both intact and castrated mice. Mammary tumors were produced in castrated female mice, but the incidence was less than that following toxic doses and less than that for intact controls.

Lymphoid tumor formation was not significantly different in controls, whether intact male or female mice or castrated males. Lymphoid tumor formation was significantly increased by nontoxic dosage of estrogen in the castrated male.

A comparison made upon litter mates, castrated at 30 days of age and receiving identical treatment as to housing, food, and dosage with estradiol, revealed 23 per cent development of tumors of the mammary gland in the castrated female against 3 per cent in the castrated male.

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Influence of Breeding on the Incidence and Onset of Carcinoma of the Mammary Gland in Marsh-Buffalo Mice

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The Marsh-Buffalo strain of mice has been used rather extensively in this laboratory in studying the influence of estrogens and gonadotropins upon the development of adenocarcinoma of the breast (1). Interest in this strain has centered upon its unique resistance to the carcinogenic activity of estrogens and its failure to respond as a high cancer strain in the laboratories of Loeb (5). Our experiments in general have been performed upon virgin mice in which the incidence of breast tumors was identical with that originally reported by Marsh (3). Because of the rather paradoxical results reported for this strain, we were prompted to study the influence of breeding upon tumor incidence under the environmental conditions existing in our laboratories.

Forty-eight mice were bred at the age of 4 months, groups of 3 females being housed with 1 male. All the females became pregnant and delivered their young in the fifth and sixth months of age. The young were weaned at 21 days of age and the mothers were again bred 15 days later. Forty-two mice became pregnant and delivered their young from the seventh to the tenth months of age. Two mice developed lymphoid tumors and 2 died of pneumonia (Table I).

The standard deviation of the mean for each tumor incidence in Table I was calculated by the formula $\sqrt{\frac{P \times Q}{N}}$, in which P is the incidence of tumors in per cent, Q denotes the mice without tumors in per cent and N is the number of animals observed. The standard error of the difference between any two incidences was taken as the square root of the sum of the squares of their respective standard deviations of the mean. A comparison of the Marsh series of virgin females and the Santa Barbara series of virgin and breeding mice reveals that the greatest difference between any of the values for a given time is 1.4 times the standard error. The probability value P of .16 for this statistic is not usually considered of significance. A comparison of the Marsh series of breeding females with the other three series of mice reveals that from the ninth to the 13th month of age, the least difference between any of the values for a given time is from 3.4 to 5.0 times the standard error. The probability value, which exceeds .001, is highly significant. From the 14th to the 18th month of age the significance of the increase in tumor incidence for the breeding mice observed by Marsh decreases, but the probability value is never below .05.

The results indicate that breeding was without influence in the mice observed in our laboratories. This was in contrast to the definite influence that the factors associated with breeding had in lowering the cancer age of the mice observed by Marsh. The observation is the more striking in view of the excellent agreement for cumulative incidence of breast tumors in virgins as observed in the two laboratories.

TABLE I: CUMULATIVE INCIDENCE * OF ADENOCARCINOMA OF THE BREAST IN MARSH-BUFFALO VIRGIN AND BREEDING MICE AS FOUND ORIGINALLY BY MARSH AND AS OBSERVED IN THE SANTA BARBARA LABORATORIES

Age, months	In Marsh's Laboratory		In Santa Barbara	
	70 Virgin	190 Breeding †	297 Virgin	48 Breeding
9	7 ± 3	36 ± 4	8 ± 2	12 ± 5
10	16 ± 4	46 ± 4	16 ± 2	15 ± 5
11	24 ± 5	58 ± 4	23 ± 2	21 ± 6
12	33 ± 6	66 ± 3	32 ± 3	31 ± 7
13	40 ± 6	72 ± 3	40 ± 3	46 ± 7
14	50 ± 6	76 ± 3	45 ± 3	54 ± 7
15	61 ± 6	80 ± 3	52 ± 3	63 ± 7
16	66 ± 6	82 ± 3	56 ± 3	65 ± 7
17	66 ± 6	86 ± 3	58 ± 3	67 ± 7
18	69 ± 6	86 ± 3	67 ± 3	70 ± 7

* In per cent ± standard deviation of the mean.
† Data reconstructed from the graph of Marsh.

It would appear that the changes in the endocrine balance associated with breeding cannot be held directly responsible for the lowering of the cancer age when it occurs. In earlier studies from this laboratory (2) it was found that appropriate dosage of gonadotropins, which produced morphological changes in the mammary gland corresponding to those produced by pregnancy, was without influence upon tumor development. These changes, initiated in the case of

the gonadotropins at the seventh month of age, occurred somewhat later than the corresponding changes in the pregnant mice. Similar results (1) were obtained with chorionic gonadotropin when dosage was begun as early as the 21st day of life.

Miller and Pybus (4) have recently published data on the influence of breeding upon tumor incidence in the Marsh-Buffalo strain. The figures they report, 68.8 per cent versus 70.4 per cent in comparing breeders with virgins, are in perfect agreement with the data from the Santa Barbara laboratories.

SUMMARY

In 48 mice of the Marsh-Buffalo strain, breeding was without influence upon the onset and cumulative incidence of cancer of the breast. This observation is in contrast to the experience of Marsh. The observations of Marsh in regard to tumor incidence in virgins have been confirmed.

Acknowledgment is made to Arthur Blanchard for the care and maintenance of the mouse colony.

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The Relative Importance of the Milk Agent and Inherited Susceptibility to It in the Development of Mammary Tumors in Mice

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During the last two decades investigators in various laboratories have developed inbred strains of mice and have studied the incidence of spontaneous mammary cancer in them, in consequence of which the following facts have emerged: (a) the occurrence of mammary tumors is remarkably constant and characteristic in each strain, both as to incidence and the age at which the neoplasms appear; (b) breeding and nonbreeding cause changes in the incidence of tumors in the females of these strains; a high incidence in the breeders of a strain does not necessarily mean that the incidence will be high also in nonbreeders of the same strain.

One of the methods used in studying the mechanism of the inheritance of spontaneous mammary tumors in mice was the reciprocal crossing in inbred strains. By observing the incidence of cancer in the first filial generation, several investigators have come to the conclusion that an extrachromosomal, nongenetic influence, in conjunction with genetic or chromosomal factors, plays an important role in etiology. Bittner (6) has further divided the genetic influences into hormonal influence and inherited susceptibility to the milk agent.

The present paper gives the incidence of mammary cancer in the first filial generation resulting from the reciprocal crossing of 2 pure strains. For comparative purposes the tumor incidence and ratios in the 2 groups were related to the tumor incidence and ratios in the 2 parent strains. In order to avoid the additional and complicating hormonal stimulation resulting from breeding, all the females in the four groups were maintained as virgins. The purpose of the study was to determine the relative significance in the 2 parent strains of the nongenetic influence (milk agent) and the genetic influence (inherited reaction or susceptibility to the milk agent). Bittner (7), in evaluating the relative importance of the 3 factors, states that "as far as can be demonstrated from the data available, the hormonal influence, the inherited susceptibility, and the milk agent are of equal importance in the etiology of mammary cancer in mice of known genetic constitution under normal conditions, and that any one of the 3 factors or influences may be completely determining in its effects." Murray and Little (9) conclude from their experiments: "Some extrachromosomal influence, which is ten times as powerful as any possible chromosomal factor, is instrumental in determining whether or not mammary cancer appears in the first out-cross generations." Murray (11) states in a later report that it might be construed from his data that the milk stimulus of one strain is 5 to 10 times more active than the other, or that the inherited resistance of the physiological system in the second strain is 5 to 10 times greater than in the first. Murray and Little made it clear that these statements cannot be, and are not intended to be, applied to all strains in general, but only to the particular strains studied by them.

The strains used in this report are the Bittner A stock and the Marsh albino. The former was obtained from Dr. Bittner in 1936 and inbred by brother-to-sister matings for 23 generations. The Marsh albinos were originally obtained from Lathrop and Loeb and bred in our laboratory for 20 years by Mr. Marsh. He referred to this strain as Strain 3, or the New Buffalo strain. Since 1936, however, this strain has been inbred by brother-to-sister matings for 24 generations and is now known as the Marsh strain. All mice received Purina fox chow and an unlimited supply of water. Henceforth the animals of the Bittner A stock will be designated as A, and those of the Marsh stock as M.

These two strains were reciprocally crossed. There were 278 F_1 hybrid females resulting from mating females of the A stock with males of the M stock (the A × M cross); 259 F_1 hybrid females resulting from mating females of the M stock with males of the A stock (the M × A cross). Simultaneously 396 females of the M stock and 207 females of the A stock were

maintained for purposes of comparison. The monthly incidence of tumors in these four groups of animals is shown in Table I. All tumors that developed were proved mammary adenocarcinoma by histological diagnosis. The percentage of mammary carcinoma and mean tumor age in months were computed, and are shown in Table II, together with the ratios of tumor incidence, using that of the Marsh virgins as 100.

TABLE I

Age, months	A ♀ × A ♂		A ♀ × M ♂		M ♀ × A ♂		M ♀ × M ♂	
	No. alive beginning of month	No. dying of cancer	No. alive beginning of month	No. dying of cancer	No. alive beginning of month	No. dying of cancer	No. alive beginning of month	No. dying of cancer
3	207	1						
4	206	0	278	1				
5	205	0	277	0				
6	205	1	276	3			396	1
7	204	1	273	7			395	0
8	203	4	266	27	259	1	393	1
9	198	1	239	31	258	3	391	5
10	197	4	208	43	255	3	385	4
11	191	2	165	39	252	2	379	19
12	187	4	126	36	250	2	358	17
13	181	11	90	31	247	6	335	17
14	163	5	59	19	241	2	310	22
15	148	3	40	7	238	7	275	31
16	135	5	33	14	230	8	232	40
17	117	4	18	6	221	4	180	25
18	103	7	11	2	214	15	146	26
19	80	3	9	3	195	12	108	17
20	67	0	6	1	177	12	79	15
21	53	1	4	0	155	16	43	7
22	43	2	4	1	134	10	28	3
23	35	1	3	1	114	8	13	1
24	29	0	1	1	91	10	0	0
25	21	0	0	0	73	3		
26	18	0			61	6		
27	11	0			47	2		
28	5	0			38	2		
31	0	0			20	1		
35					5	1		
38					1	1		
Total		60		273		137		251

TABLE II

	A ♀ × A ♂	A ♀ × M ♂	M ♀ × A ♂	M ♀ × M ♂
No. of mice in each group	207	278	259	396
No. of mice developing cancer	60	273	137	251
Observed % of mammary cancer	29.0	98.0	53.0	63.5
Ratio	45.7	154.2	83.5	100.0
Mean tumor age (months)	14.06	11.47	19.56	15.53

DISCUSSION

It can be observed that the tumor rate in the F_1 hybrids of the A × M cross is considerably higher than the rates for the reciprocal cross M × A and the virgins of the A or the M stock. From the fact that the cancer incidence in the A × M cross is higher than that of either of the parent stocks and that the mean tumor age was distinctly lower, we might conclude that this is the result of the combination of a high concentration of the extrachromosomal, or milk, agent and a high degree of susceptibility to it. Since the milk agent could only come from the A stock female of this cross, we have tentatively assigned a high concentration of this extrachromosomal stimulant to the females of the A stock. The high susceptibility of these hybrids may have come from either the A stock female or the M stock male or from both. However, if the A stock, with its high concentration of the milk agent, was also highly susceptible one would expect a high rate of cancer in the virgins of this strain. But the experimental results indicate that the cancer rate in

the virgins of this stock is lower than in the virgins of the Marsh stock. Therefore we conclude that the inherited reaction or susceptibility of the A stock to the stimulation of the milk agent must be lower than that in the Marsh strain.

Applying the conclusions arrived at in the preceding paragraph to the reciprocal cross, M × A, we have a high inherited (genetic) susceptibility contributed by the Marsh female and a low inherited (genetic) susceptibility contributed by the A stock male. We are now ready to determine the concentration of the milk agent in the Marsh stock females. Since the cancer rate of the M × A cross is low, it must follow that the concentration of this agent in the Marsh females is lower than that of the A stock females.

The M × M group of mice, according to these observations, must therefore have a high inherited susceptibility and a relatively low concentration of the extrachromosomal agent. Their cancer incidence, however, is higher than that of the M × A and the A × A groups. Since we have assumed a high concentration of the milk agent and a low inherited susceptibility to this agent for the A × A group, and the cancer incidence is low in the virgins; and also since we estimate the reverse, or low concentration of the milk agent and high inherited susceptibility, to be true for the M × M group yet the cancer rate of the virgins is high, we can only conclude that the inherited susceptibility is more important in determining the development of mammary cancer in these 2 strains of mice than is the concentration of the milk agent. Any further quantitative estimation of the concentration of the milk agent or the inherited susceptibility of the 2 strains is not possible from these data. Further studies involving other strains of mice are in progress. We believe that in determining the quantitative relation between extrachromosomal or milk agent and the inherited susceptibility to that agent, it would be of value to establish the cancer rate in virgins of these strains foster-nursed after cesarean section by mothers of the opposite strain, and also by mothers of a third strain. This work is also in progress.

SUMMARY

Tumor rates of 4 groups of virgin mice, Marsh, A stock, and the reciprocal crosses of the 2 strains were computed and are presented in tabular form. The A × M hybrids showed a higher tumor rate than either of the parent stocks, and approximately double that of the reciprocal cross. It can be concluded from these experiments:

1. The extrachromosomal, or milk, agent is more concentrated in the A than in the M stock.

2. The M stock is genetically more susceptible to the milk agent than the A.

3. The inherited susceptibility of the physiological system is of greater importance than the milk agent in the development of mammary tumors in these 2 strains of mice.

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THE VASCULAR REACTION OF THE MOUSE TO NORMAL AND NEOPLASTIC TRANSPLANTS AND WOUNDS. GLENN H. ALGIRE, and HAROLD W. CHALKLEY. (National Cancer Institute, Bethesda 14, Md.)

Microscopic studies by *in vivo* methods were made of the vascular reaction of the mouse to wounds, and to implants of normal and neoplastic tissue. The wounds or implants were made in tissue included within a transparent chamber introduced into a dorsal skin flap in the mouse. Photographs and daily quantitative measurements were made of the vascular levels in the surrounding normal tissue and in the implant or wound site.

The initial stages were similar in all. There was an accumulation of leucocytes in the involved area, followed by capillary dilatation, and new capillary proliferation at about the fourth to sixth day. The vascular levels in aseptic wounds attained the level of the normal subcutaneous connective tissue that was used as a base line, then stabilized. Implants of adult liver and spleen attained a vascular level double that of the connective tissue, then subsided to the base line. Tumor implants rapidly attained a vascular level double that of the connective tissue, and maintained this level during the period of observation (15 to 25 days). Tumor vessels originated as capillaries and remained so. Capillaries in wounds and about implants of adult tissues differentiated into arterioles and venules. Evidence is presented that tumor tissue possesses the property of *continuously* eliciting new capillary endothelial growth from the host and thus establishing and maintaining a rich nutritive supply. It is suggested that this property, rather than some vague "autonomy" of the tumor cell is, from the standpoint of the host, an important expression of the neoplastic change.

RELATION OF GENES TO MAMMARY TUMOR DEVELOPMENT IN THE MOUSE. W. E. HESTON. (National Cancer Institute, Bethesda 14, Md.)

It is now accepted that genic differences can vary the probabilities that a mouse will develop a mammary tumor. From recent work on gene chemistry it is reasonable to expect that these genic differences can be manifested as enzymatic differences affecting specific metabolic reactions leading to the development of the tumor. Although the identification of the specific genes and of the specific reactions with which each is concerned are probably still far in the future, we have accumulated evidence that points to the general fields in which at least certain of these genetic differences are manifested.

There is evidence that genic differences can result in variation in response of the mammary tissue to the hormonal and/or the milk agent stimulation. In this case the primary gene action may occur in the mammary tissue cell.

Experiments have shown that certain genic differences are manifested as variation in the hormonal stimulation. Here one can hardly predict where the primary gene action occurs other than that it is probably within the endocrine system.

Experimental results have shown that certain genic differences can affect variation in the propagation and transmission of the milk agent. Since the milk agent has been demonstrated in various organs and tissues there is no evidence that in this case the primary gene action is limited to any particular tissue. Such gene action not only may influence the development of the mammary tumor of the individual but by affecting variation in the transmission of the agent also may cause variation of tumor development in the daughters or foster-nursed females.

This demonstrated intimate interrelation between the chromosomal factors and the milk agent presents a concept in many respects not unlike the gene-cytoplasm relationship concept of normal differentiation involving the cytoplasmic determiners plasmagenes and plastogenes.

TRANSPLANTATION OF SPONTANEOUS PRIMARY HEPATOMAS IN MICE OF STRAIN C3H. EDWARD L. BURNS, and JOHN R. SCHENKEN. (Department of Pathology and Bacteriology, Louisiana State University School of Medicine, New Orleans 13, La.)

Transplantations of spontaneous primary hepatomas arising in 3 C3H strain male mice were made into 28 young C3H mice as follows:

Tumor tissue was transplanted subcutaneously into 11 mice, intraperitoneally into 4, and into the anterior chamber of the eye in 4; a saline emulsion of tumor tissue was injected into the anterior chamber of the eye in 5 mice; sterile filtrates of tumor tissue were given intradermally, subcutaneously, and intrahepatically to 1, 1, and 2 mice respectively.

The transplants grew in 1 of the 11 mice inoculated subcutaneously, and in 3 of the 4 animals inoculated intraperitoneally. No tumors grew in the animals of any other group. The transplanted tumors were found from 9.1 to 20.5 months after the transplants were made. They formed solid, rounded, lobulated masses measuring from

1 to 2 cm. in diameter, and were either red or yellow in color. Microscopically they closely resembled the primary neoplasms.

THE INFLUENCE OF CALORIC RESTRICTION ON TUMOR FORMATION AND ON THE LEVEL OF BLOOD SUGAR IN MICE. H. P. RUSCH, V. R. POTTER, and R. O. JOHNSON. (McArdle Memorial Laboratory, Medical School, University of Wisconsin, Madison 6, Wis.)

The appearance of neoplasms can be delayed and the incidence of tumors reduced when experimental animals are given a diet restricted in calories. Although the mechanism of tumor inhibition caused by decreased caloric intake is unknown, there is some indication that neoplastic cells fail to proliferate until the nutritive energy rises above a critical level. To test this theory, the level of blood sugar was determined in mice receiving either high or low calorie diets of various compositions, and the results were compared with the incidence of tumor formation in the various groups. The mice were given a single subcutaneous injection of 200 μgm. of 3,4-benzpyrene dissolved in corn oil. The groups on the restricted calories received a calculated 5.5 calories per mouse per day, and the other groups an average of 9 calories per mouse per day. Tumors were observed from 2 to 3 weeks earlier in the mice on the high calorie diets, and the tumor incidence in these groups at 4 months was 85 per cent as compared to 40 per cent for the mice restricted in calories. With the exception of 1 group the mice on high calorie diets had higher levels of blood sugar (125 to 150 mgm. per cent) than those on the restricted diets (90 to 100 mgm. per cent). One group receiving a high caloric intake, though carbohydrate was largely replaced by increased amounts of casein and fat, showed the same rate of tumor formation as the other groups on the same caloric intake, but at a level of blood sugar that was only slightly higher than was observed in the mice on restricted calories.

CASTRATION EFFECTS IN RELATION TO THE INHERITED HORMONAL INFLUENCE IN MICE. FERN W. SMITH, and JOHN J. BITTNER. (Department of Physiology, Division of Cancer Biology, University of Minnesota Medical School, Minneapolis 14, Minn.)

Female mice of the high cancerous A and C3H stocks and 2 groups of their reciprocal hybrids, 1 with and 1 without the active milk agent, were castrated at time of weaning and observed for evidence of hormonal stimulation.

At 7 months normal A strain virgins have as high an occurrence of estrous cycles as do normal C3H virgins. Among castrates of the same age there is a complete lack of estrus in A strain mice in contrast to the high value, comparable to the normal, observed among the C3H and hybrid mice. As Woolley, Fekete, and Little noticed in their C3H and dba stocks, the C3H and, in addition, the hybrid castrates showed gross and microscopic hyperplasia of the adrenal cortex. Uterine and mammary gland development with tumors followed in the C3H animals and the hybrids with the milk agent. Those hybrids lacking the milk agent, in spite of adrenal changes, showed no precancerous mammary lesions or tumors. Animals of the A strain failed to show the indication of hormonal stimulation mentioned above.

The lack of the inherited hormonal factor is partially responsible for the low tumor incidence in normal virgins of the A stock. Virgin C3H mice possessing this factor have a much higher incidence. Since the adrenal changes appeared only in those animals with the inherited hormonal influence, it seems probable that the changes among the castrate animals of those strains and their reciprocal hybrids may be dependent upon this character. The presence of the milk agent, however, is essential for tumor development among the hybrids. The A strain castrates, lacking the inherited hormonal influence, may show modified mammary and adrenal growth but no other evidence of hormonal stimulation.

GENETIC FACTORS IN THE ETIOLOGY OF MAMMARY CANCER IN MICE. JOHN J. BITTNER. (Department of Physiology, Division of Cancer Biology, University of Minnesota Medical School, Minneapolis 14, Minn.)

Observations on reciprocal hybrids between the high cancer A and the low cancer B (C57 black) stocks demonstrated that the mice of both sexes of the former strain transmitted the inherited susceptibility for spontaneous mammary cancer. For the expression of the susceptibility, both the milk agent and hormonal stimulation of breeding were necessary.

When breeding females of the high cancer A and C3H stocks and their reciprocal hybrids were studied, the incidence observed in hybrids of the F_1 and F_2 generations was comparable to that of the parental stocks. These data suggested that either the genes producing the inherited susceptibility in the two high cancer stocks were the same or, if different genes were involved, any combination of dominant genes would produce susceptibility in the F_2 hybrids.

Whereas the virgin females of the A stock have a low incidence, virgin females of the C3H stock and the reciprocal hybrids between the A and C3H stocks frequently develop mammary cancer. Thus the mice of the C3H stock transmit, in addition to the genes producing the inherited susceptibility for mammary cancer, genes that determine whether or not virgin females will give rise to this type of cancer. This character, now being called the inherited hormonal influence, is another inherited condition to be considered in the etiology of mammary cancer, at least in virgin animals.

ANTIGENIC CHARACTER OF THE CANCER MILK AGENT IN MICE. ROBERT G. GREEN, MARYE M. MOOSEY, and JOHN J. BITTNER. (Department of Bacteriology and Immunology and the Department of Physiology, Division of Cancer Biology, University of Minnesota Medical School, Minneapolis 14, Minn.)

Immunological studies of the cancer milk agent in mice are being made by the utilization of high-speed centrif-

ugates of cancer tissue suspensions as the infective agent. The centrifugates, collected between 15,000 and 95,000 gravity, were previously demonstrated to contain the milk agent. Such centrifugates have been injected into rabbits to produce antiserum. Rabbits were given 5 injections of centrifugate at 5 day intervals, each dose representing 4 gm. of spontaneous mouse tumor suspended in 3.5 cc. of saline solution. The rabbits were bled for the production of antiserum 9 and 10 days after the last injection.

A group of 30 mice was injected with a centrifugate equivalent of 0.25 gm. of tumor tissue suspended in 0.5 cc. saline solution. At the end of $8\frac{1}{2}$ months, 24 of the 30 mice have developed mammary cancer. Thirty mice were injected with a similar amount of a centrifugate equivalent of 0.25 gm. of tumor tissue suspended in 0.5 cc. of rabbit immune serum, the mixture being allowed to stand at room temperature 2 hours before injection. The antiserum was a pooled mixture of the sera of 3 rabbits immunized as described above. At $8\frac{1}{2}$ months, 2 of the 30 mice have developed tumors. Of 30 mice injected with similar amounts of centrifugate suspended in 0.5 cc. of normal rabbit serum, 5 have developed mammary cancer at $6\frac{1}{2}$ months.

These results, although reported after a relatively short time, would seem to indicate that the milk agent stimulates antibody production and therefore is antigenic. This character, combined with previously demonstrated characters of the milk agent, those of ultramicroscopic size and reproduction in association with living cells, would seem to identify the milk agent of mouse carcinoma as a filterable virus.

NET RESISTANCE AGAINST TUMOR GROWTH: COMBINED EFFECT OF ESTROGENS, MILK FACTOR, AND GENETIC FACTOR. WILLIAM T. SALTER and L. MURIEL SCHLEGEL. (Department of Pharmacology, Yale University, New Haven 11, Conn.)

When the growth of implanted sarcoma 180 was studied in pedigreed mice, resistance was most frequent under the following circumstances: (a) The host animal normally showed a *low* incidence of spontaneous tumors; (b) the source of the implanted tumor was a *low* tumor substrain; and (c) preliminary "immunity" was produced by inoculation (in the tail) of tumor raised in a *low* tumor substrain. The incidence of takes was highest when these features were reversed. Intermediate combinations gave intermediate results.

The studies emphasize the complex nature of susceptibility toward tumor growth. They indicate a partial analysis of such resistance. They suggest that, quite independently of the origin of neoplasms (carcinogenesis), somatic factors may be varied so as to retard neoplastic growth.

RELATIVE THIAMINE DEFICIENCY IN CANCEROUS INDIVIDUALS. RIGBY C. ROSKELLEY, L. MURIEL SCHLEGEL, and WILLIAM T. SALTER. (Department of Pharmacology, Yale University, New Haven 11, Conn.)

There is a high incidence of thiamine deficiency in cancer patients encountered in a general hospital. This incidence is considerably greater than in patients suffering from other diseases. When the thiamine tolerance of cancer patients is tested, they show a diminished ability to store *free* thiamine. If a large dose of thiamine is administered the peak of urinary excretion is frequently reached at the third hour. Thereafter, thiamine excretion falls in approximately logarithmic fashion. By appropriate calculations an index of the free thiamine store can be established for comparative purposes. Part of the effect is attributable to the incidence of cancer in the higher age brackets. Even so, there remains evidence of a relative thiamine deficiency in certain cancer patients who have previously been flooded with thiamine.

ATTEMPTS TO PRODUCE CANCER IN RHESUS MONKEYS WITH CARCINOGENIC HYDROCARBONS AND ESTROGENS. CARROLL A. PFEIFFER, and EDGAR ALLEN.* (Department of Anatomy, Yale University, New Haven 11, Conn.)

The treatment of monkeys (*Macacus rhesus*) used in cancer experiments from 1936 to the present is summarized below.

No. of monkeys *	Chemicals **	Period of treatment, years	Dosage		Injections in oil ***	Painted in benzene ***	Pellets
			Weekly, mgm.	Total, mgm.			
17	MC	$\frac{1}{2}$-$8\frac{1}{2}$	2-6	26-1500	+	+	+
10	BP	$\frac{1}{2}$-8	2-4	28-690	+	+	+
6	DBA	$\frac{1}{2}$-$8\frac{1}{2}$	2-4	18-450	+	+	
16	E	$\frac{1}{2}$-5	variable	0.0085-591	+		+

* Many animals received a combination of chemicals. Monkeys treated less than $\frac{1}{2}$ year are not included.
** MC = methylcholanthrene, BP = benzpyrene, DBA = dibenzanthracene, and E = estrogens.
*** Control animals were treated with benzene and sesame oil for 1 to 2 years.

Almost all organs and tissues that frequently become cancerous in the human subject were treated, including the abdominal and pelvic viscera. The mammae received the longest treatment, although multiple sites were used in all animals. Carcinogens were also injected intravenously and administered orally. Methylcholanthrene and dibenzanthracene caused more pronounced local reactions than did benzpyrene. Following injections in oil these consisted of firm non-inflammatory fibrotic masses, areas of granulation tissue, and encapsulated cysts of the injected material. Following painting they consisted of hyperkeratinization

* Deceased.

with loss of hair, cystic hair follicles, cystic sebaceous glands, and papillatomatous masses or wart-like excresences. Pellets became encapsulated in dense fibrous connective tissue surrounded by numerous round cells and phagocytes. This reaction sometimes progressed until distinct, firm, noninflammatory, fibrotic masses were produced. The lesions just described might persist, regress, or become infected and ulcerate. Metaplasia of the mammary ducts and of the epithelium of the cervical glands occurred. Malignant tumors were not found.

HORMONAL FACTORS IN THE GROWTH OF TRANSPLANTED TESTICULAR TUMORS IN MICE. W. U. GARDNER. (Department of Anatomy, Yale University, New Haven 11, Conn.)

Testicular interstitial cell tumors occur in estrogen-treated mice of some strains (observations reported previously by several investigators). In our laboratory these tumors have grown, subsequent to transplantation, only in estrogen-treated mice. Two different testicular tumors were transplanted for 4 and 9 generations. One arose in a hybrid mouse (A×C3H) and one in an A strain mouse. Both primary neoplasms metastasized to lumbar or perirenal nodes, and histologically resembled tumors previously described.

The tumors were grafted into genetically related or unrelated mice of inbred strains, or into hybrid groups. Stilbestrol, in either oily solution (0.25 mgm. weekly) or compressed pellets (4 to 8 mgm., 1 part stilbestrol and 3 parts cholestrol) was the estrogen used.

(A) The tumors grew (1) only in genetically related mice and (2) only in such mice given estrogens.

(B) The transplants in untreated, genetically related mice regressed; they were not detected or were found only when sought under ultraviolet light (bright orange fluorescence).

(C) The tumor 'rests' mentioned under (B) were capable of growth up to 7 months subsequent to transplantation if estrogen treatment was inaugurated. Histologically the 'rests' contained predominately macrophages and few or no recognizable tumor cells.

(D) When growth had started during a period of estrogen treatment the tumors persisted or continued to grow subsequent to cessation of treatment.

(E) The tumors persisted subsequent to hypophysectomy although the hosts lost weight.

(F) The transplants in different animals grew at variable rates, and contained various amounts of myelopoietic and hematopoietic cells.

SPONTANEOUS INTERSTITIAL CELL TUMORS OF THE TESTIS IN DOGS. CHARLES W. HOOKER, CARROLL A. PFEIFFER, and JOSEPH DEVITA. (Department of Anatomy, Yale University, New Haven 11, Conn.)

An attempt has been made to compare spontaneous tumors of the interstitial cells of the testes of dogs with tumors of the same cells in estrogen-treated mice of the A strain. To this end the testes of 38 old dogs of several breeds have been studied microscopically. Seven interstitial cell tumors were found. As in the estrinized mice, the tumor masses were well circumscribed and contained many engorged vascular spaces. The tumor cells were like "second generation" tumor cells of the mice, except in one tumor that was composed of cells resembling those of the "second" and "third generations." Small nodules of interstitial cells like the earliest tumors of the mice were present in 6 testes. The intertubular tissue exhibited no remarkable feature in 9 testes. In 14 testes and in the nontumorous portion of the 13 testes with small or large tumors the intertubular tissue was like that described many years ago by Goodpasture and Smith. The Leydig cells were hypertrophied, highly vacuolated, and contained large cytoplasmic granules. Many were in various stages of disintegration. Among the Leydig cells were many cells containing a yellow pigment. These are structural features regularly present in the testes of A strain mice in the early months of treatment with estrogen. Apparently, therefore, the spontaneous tumors of the dogs resemble those arising in estrogen-treated A strain mice with respect to structure and to developmental stages.

THE DEPENDENCE OF TUMOR FORMATION ON THE DEGREE OF CALORIC RESTRICTION. ALBERT TANNENBAUM. (Department of Cancer Research, Michael Reese Hospital, Chicago 16, Ill.)

In previous publications it has been reported that caloric restriction results in an inhibition of tumor formation in the mouse. This effect has been demonstrated for spontaneous mammary and lung tumors, for induced sarcomas and epitheliomas, and for leukemias. In general, the caloric intake of restricted animals was approximately 60 per cent or less of the *ad libitum* intake. In the present experiments, which were performed with spontaneous mammary and induced skin tumors, the caloric intakes were graded. The diets for the various groups differed only in their carbohydrate content, and ranged in caloric value from approximately 60 per cent to 90 per cent of the *ad libitum* diet. The results of the experiments indicate that any degree of caloric restriction may exert some inhibitory effect on the formation of the two types of tumors studied. The inhibitory effect does not appear to be directly proportional to the degree of caloric restriction, tending rather to show upper and lower limits.

THE DEPENDENCE OF TUMOR FORMATION ON THE COMPOSITION OF THE CALORIE-RESTRICTED DIET AS WELL AS ON THE DEGREE OF RESTRICTION. ALBERT TANNENBAUM. (Department of Cancer Research, Michael Reese Hospital, Chicago 16, Ill.

The inhibitory effect of caloric restriction on the formation of tumors in mice is dependent on the actual degree of this restriction. The present experiments, which employed the spontaneous mammary and the induced skin tumor, were performed to confirm this finding and also to ascertain the significance of the composition of the diet at any particular level of caloric restriction. Three types of restricted diets were utilized: (a) diets in which the caloric restriction was achieved by limiting the carbohy-

drate component only; (b) diets in which caloric restriction was achieved by limiting the amount of the control ration (all components restricted proportionately); and (c) diets equivalent to the latter except that they were high in fat content.

The results with all three types of diet confirm the introductory statement. The results also suggest that caloric restriction achieved by limiting only the carbohydrate component of the diet may be more effective in inhibiting tumor formation than is caloric restriction achieved by restricting all components of the diet. Furthermore, it is demonstrated that the augmenting effect of a high fat diet on the formation of tumors occurs at all levels of caloric restriction studied. This augmenting effect is mediated through some specific action of the ingested fat.

PHOSPHOLIPIDS IN HUMAN NEOPLASMS. KENNETH W. BUCHWALD, and LEONA HUDSON. (State Institute for the Study of Malignant Diseases, Buffalo 3, N. Y.)

The phospholipids of tumor cells and nuclei were isolated from autopsy specimens. The nuclei were first separated from the tumor cells by the procedure of Stoneburg. The phospholipids in the nuclei and whole tumor tissue were isolated according to Bloor's procedure. In the phospholipid fraction the phosphorus was determined by the method of Kuttner and Lichtenstein, and the choline according to Haven's modification of Beattie's procedure. To date 8 neoplastic growths have been examined. The phospholipids varied over a wide range. Whole tissue gave a maximum of 7.59, minimum 1.06, and mean 3.46 gm. per 100 gm. dry tissue. Nuclei gave a maximum of 9.70, minimum 2.88, and mean 6.47 gm. per 100 gm. dry nuclei. In all specimens the phospholipid in the nuclei was higher than in the whole tissue. The analysis for choline in whole tissue gave a maximum of 0.56, minimum 0.20, and mean 0.37 gm. per 100 gm. dry tissue and in nuclei a maximum of 1.33, minimum 0.27, and mean 0.80 gm. per 100 gm. dry nuclei. The higher choline content of phospholipids in the nucleus indicates a larger proportion of lecithin in the nucleus than in the whole cell. The molar ratios of choline to phosphorus indicate that the cephalin content varies more than the lecithin and sphingomyelin. This ratio was higher in the nuclei in all specimens except one (carcinoma of the prostate). Although the molar ratios varied for each tissue analyzed they were of the same magnitude in both nuclei and whole cells of the same specimen. From the data thus far collected it is evident that variations in the phospholipid content of human neoplasms is produced by changes in the cephalin fraction rather than in the lecithin or sphingomyelin.

THE PHOTOMETRIC STUDY OF NUCLEIC ACIDS IN HUMAN TUMORS. ROBERT E. STOWELL. (Department of Pathology, Washington University School of Medicine, St. Louis 10, Mo., and the Barnard Free Skin and Cancer Hospital, St. Louis 3, Mo.)

The nucleoproteins and their constituent nucleic acids have important vital functions in normal cells. There is increasing evidence that the nucleic acids of the nucleus and cytoplasm are altered in some malignant cells.

The relative amounts of nucleic acids of the ribose and desoxyribose type have been determined in a series of human tissues, and the measurements on various normal and neoplastic tissues have been compared. The histochemical measurements were made with a special photometric instrument consisting of a light source, light filters, microscope, photocell, and amplification and recording apparatus. The thymonucleic acid was measured by determining the absorption of monochromatic light in tissues stained by the Feulgen reaction. Ribonucleic acid was estimated by photometrically determining the decrease in staining of cells after treatment with the enzyme ribonuclease, which splits ribonucleic acids. The results are related to the amounts per unit volume of tissue and per cell. As compared with the corresponding normal tissues, statistically significant increases in the amounts of thymonucleic acid per unit volume of tissue were found in an adenocarcinoma of the rectum, a primary carcinoma of the liver, an epidermoid carcinoma of the epiglottis, and a leiomyosarcoma. The amounts of the thymonucleic acid per cell were significantly increased in the primary carcinoma of the liver and adenocarcinoma of the bronchus. An adenocarcinoma of the prostate and a leiomyosarcoma contained increased amounts of ribonucleic acid per unit volume of tissue and per cell. The measurements are being continued.

THE CATHEPTIC ACTIVITY OF NORMAL AND NEOPLASTIC MOUSE TISSUES. MARY E. MAVER, and THELMA DUNN. (National Cancer Institute, Bethesda 14, Md.)

The catheptic activities of several spontaneous and induced mouse neoplasms were compared, and the effects of these neoplasms upon the catheptic activities of the liver, spleen, and lungs of the tumor-bearing mice were studied. The activities were determined by the quantity of tyrosine liberated in 10 minutes by the proteolytic action of an extract of the tissues on a solution of hemoglobin at pH 3.5. The activities of extracts of spontaneous mammary carcinomas of the A and C3H strains and the first transplants of these tumors were greater than the activities of tumors that have been transplanted over a period of years—such as the Crocker sarcoma 180, sarcoma 37, and the Taylor mammary carcinoma. The spontaneous tumors also showed more catheptic activity than the transplants of induced tumors, including the hepatomas induced by injecting 2-amino-5-azotoluene or carbon tetrachloride, and the intestinal adenocarcinoma induced by giving methylcholanthrene in the drinking water.

The livers and spleens of mice bearing some of the neoplasms were augmented in size and had increased proteolytic activity. These changes were not always directly related to the size of the tumors, since some smaller spontaneous neoplasms caused more change than many of the larger transplanted tumors. An attempt has been made to correlate these changes in catheptic activity in the liver and spleen with the degree of extramedullary hematopoiesis and the number of lymphocytes present in these tissues,

since lymphoid tissue, as represented by the spleen and thymus, showed the greatest normal catheptic activity. The transplants of spontaneous and induced leukosis, which kill rapidly by the infiltration of leukemic cells, also produced the most rapid increase of catheptic activity in the organs of mice with these leukoses.

INDUCTION OF PULMONARY TUMORS IN MICE BY ANESTHETIC AGENTS. C. D. LARSEN, and W. E. HESTON. (National Cancer Institute, Bethesda 14, Md.)

Strain A and strain C mice injected intraperitoneally with narcotizing doses of a series of carbamic acid derivatives exhibited large numbers of pulmonary tumors when necropsied at 6 months of age. Ethyl carbamate (urethane), when administered repeatedly on a dose/weight basis, incited the formation of approximately 50 per cent more lung tumors in male than in female mice; when administered in equal amounts to male and female mice the difference in number of lung nodules in the sexes was largely eliminated. Appreciable, though smaller, numbers of nodules were found after repeated injections of divided doses. Necropsy of mice at monthly intervals following treatment revealed that the average tumor count per animal approached a maximum in about 3 months.

Propyl, butyl, and iso-amyl carbamates proved relatively ineffective. Derivatives of ethyl carbamate in which either or both of the amino hydrogen atoms were substituted by aliphatic groups exhibited a variable and modified effect.

Anesthesia, *per se,* appeared to be unrelated to the tumor inciting phenomenon. Intraperitoneal administration of narcotizing doses of ethyl alcohol, chloral hydrate, or paraldehyde, under the conditions of these experiments, elicited no significantly greater number of lung tumors than were found in control mice. Twelve of the more commonly used barbituric acid derivatives were without effect.

EFFECT OF NUCLEATES ON DEHYDROGENASES IN LIVER AND HEPATOMA. JESSE P. GREENSTEIN, and HAROLD W. CHALKLEY. (National Cancer Institute, Bethesda 14, Md.)

Tissue extracts possess the capacity of decolorizing methylene blue under anaerobic conditions. The addition of xanthine to such extracts results in an increase in the decolorization rate when the relative proportion of dye to substrate is high (measure of xanthine dehydrogenase activity). When the relative proportion of dye to substrate is very low, the addition of the latter results in a retardation of decolorization rate (apparent negative xanthine dehydrogenase activity). This holds true for extracts of both normal liver and hepatoma.

The addition of sodium desoxyribosenucleate to extracts containing high concentrations of dye results in retardation of decolorization of uniform extent whether xanthine is present or not; therefore under these conditions the xanthine dehydrogenase activity is relatively little affected. On the other hand, in the presence of very low concentrations of the dye, the presence of the nucleate results in an acceleration of decolorization when xanthine is added, yielding not only a positive dehydrogenase activity but in greater magnitude than in the presence of high concentrations of dye. This effect is less definite in hepatoma extracts than in extracts of liver.

SUNLIGHT AS A CAUSAL FACTOR IN CANCER OF THE SKIN. HAROLD F. BLUM.* (Naval Medical Research Institute, Bethesda 14, Md.)

Recent epidemiological studies by Dorn indicate a definite north-south distribution of cutaneous cancer incidence in the United States, the numbers being greater in the south. The incidence of internal cancer does not follow this distribution. These data have been compared with the distribution of annual insolation over the same latitude range by (A) total sunlight, (B) those ultraviolet wave lengths that induce tumors in mice and rats.

(A) varies so little with latitude that it cannot be considered as a cause of cutaneous cancer. (B) varies somewhat less with latitude than does cutaneous cancer incidence.

If (B) is assumed to be the causal factor in human cutaneous cancer, the difference in variation with latitude may be accounted for on the basis of seasonal changes in (B), if certain experimental findings are taken into account. Experiments on mice show that below a certain intensity the carcinogenic effectiveness of (B) falls off sharply, and that development of tumors is greatly dependent upon interval between exposures to (B) rest periods permitting some recovery. During the winter months the carcinogenic action of sunlight should be greatly reduced because of the low intensity of (B), and hence the winter season constitutes a period of rest from exposure that increases in length with latitude. The hypothesis that sunlight or, more correctly, a portion of sunlight (B), is the major causal factor in human cutaneous cancer is supported by its geographical distribution as well as by much other converging evidence both clinical and experimental.

THE EXCISION OF CANCER UNDER MICROSCOPIC CONTROL BY THE CHEMOSURGICAL METHOD. FREDERIC E. MOHS. (Department of Surgery, and McArdle Memorial Laboratory, Medical School, University of Wisconsin, Madison 6, Wis.)

The systematic microscopic control of excision is the most important and the only completely novel feature of the chemosurgical method of cancer therapy. This microscopic control is accomplished by a process consisting of the following steps: (a) chemical fixation of the suspected tissues *in situ* by the application of a zinc chloride fixative paste; (b) surgical excision of a layer of fixed tissue, which is divided into specimens the locations of which are mapped on the lesion and on paper; (c) microscopic examination of frozen sections through the under surface of each excised specimen. The areas of cancer so located are subjected to a repetition of the process until a cancer-free plane is reached.

* The opinions and views contained herein are those of the writer and are not to be considered as reflecting the views of the Navy Department.

The microscopic control confers the advantages of unprecedented reliability and conservatism. The unsuspected outgrowths that commonly extend out from the main cancer mass and constitute a not uncommon cause of failure by surgical or radiation procedures are systematically followed out and eradicated by the chemosurgical technic. Many cancers that are inoperable because of extent or difficult position, and cancers resistant to x-ray or radium therapy, are still amenable to chemosurgery.

The method is limited to tumors that are accessible because the precedure is carried out in stages. Special training and specially equipped clinics are desirable.

SPONTANEOUS AND INDUCED GASTRIC CARCINOMA IN MICE. LEONELL C. STRONG. (Department of Anatomy, Yale University, New Haven 11, Conn.)

Four types of malignant gastric tumors have appeared in mice of the NHO descent following the subcutaneous injection of 1 mgm. of methylcholanthrene dissolved in 0.1 cc. of sesame oil at 60 days of age. These are (a) squamous carcinoma of the forestomach; (b) adenocarcinoma derived from the gastric mucosa; (c) adenoacanthoma; and (d) leiomyosarcoma. These tumors appeared only in hybrid mice selected genetically toward the suppression of the usually occurring local tumors such as fibrosarcoma and epidermoid carcinoma. Two selected sublines of the NHO have so far been continued for several generations; one is characterized by a very high incidence of gastric carcinoma following the subcutaneous injection of the methylcholanthrene. Mice of the other selected subline give rise to adenoacanthoma following the same technic.

At 6 month intervals mice have been weaned from the group in the first subline and kept under normal laboratory conditions. The incidence of spontaneous gastric carcinoma is increasing rapidly in the successive groups. At the present time an incidence between 95 and 100 per cent of spontaneous gastric carcinomas at 420 days of life is being obtained. The mice derived from methylcholanthrene-injected parents transmit the condition through at least 3 (untreated) generations. Linkage evidence available indicates that the susceptibility to induced gastric carcinoma in the parents is hereditarily determined.

PRELIMINARY STUDIES ON THE AUTONOMY OF THE ROUS SARCOMA AS MEASURED BY GROWTH OF THE TUMOR IN THE ANTERIOR CHAMBER OF GUINEA PIG EYES. E. W. SHRIGLEY. (Yale University School of Medicine, New Haven 11, Connecticut)

The Rous sarcoma grows in the anterior chamber of guinea pig eyes, as is shown by the fact that small grafts placed in this environment become vascularized, increase in size, and show mitotic figures. Unlike mammalian cancer in the anterior chamber, however, this vascularization takes place early, within 3 or 4 days, and maximum growth is reached in 2 or 3 weeks, after which regression occurs. No tumor has been observed to reach such a size as to fill the chamber completely. Moreover, serial transfer in the eyes of guinea pigs has proved unsuccessful beyond 2 passages. Evidence that the tumor cells survive in the eye is based not only on the observations mentioned above, but also on the fact that chicks develop sarcomas when inoculated with tumor that has been in the guinea pig eye for 10 or 12 days. The virus is likewise known to be present in these transplants, since birds inoculated with tumor tissue from the eye show hemorrhagic, nonneoplastic lesions in the liver and other organs that are characteristic of the Rous virus infection in individuals of low resistance.

As soon as the Rous sarcoma becomes visible in the chick it will grow when transplanted into guinea pig eyes. On the other hand, virus-infected chicken tissue showing no macroscopic growth has not as yet, following residence in the guinea pig eye, produced tumors in chicks. Whether sarcomas that have persisted in birds for long periods of time are still transplantable to guinea pigs is yet to be determined.

Two other virus-induced chicken tumors have been grown in the anterior chamber of guinea pig eyes. Breast muscle of normal 2 day old chicks gives no evidence of growth in this environment.

SENSITIZATION AS THE FIRST PHASE IN SKIN CARCINOGENESIS. TRAUMA AND CANCER. W. CRAMER, and W. L. SIMPSON. (Barnard Free Skin and Cancer Hospital, St. Louis 3, Missouri)

Repeated application over a long period of a chemical carcinogen dissolved in lanolin to the skin of mice fails to produce any of the chemical, histological, and cytological changes that follow a single application of the carcinogen dissolved in benzene. But while the skin does not show any demonstrable change from the normal it has undergone a lasting biological alteration in so far as it has been sensitized to the action of the carcinogen in benzene. This sensitization is, however, not specific for one particular carcinogen. The application of a single nonspecific trauma such as a burn to a "sensitized" skin induces cancer. (a) Sensitization is the first phase in the reaction of the skin to contact with a chemical carcinogen, inducing an increased susceptibility of the treated skin to the development of cancer. (b) Susceptibility can be acquired, and is not necessarily a fixed genetic factor. (c) The two main factors concerned in carcinogenesis, carcinogenic stimulus and susceptibility, may be linked together more closely than hitherto supposed as manifestations of the same agent. (d) The carcinogenic potentiality of a single nonspecific trauma has been established on an experimental basis.

FURTHER STUDIES ON THE RELATION BETWEEN RADIATION EFFECTS, CELL VIABILITY, AND INDUCED RESISTANCE TO MALIGNANT GROWTH. III. INDUCED RESISTANCE IN RATS OF AN INBRED STRAIN (HOMOZYGOUS) TO A LYMPHOSARCOMA THAT ORIGINATED IN THE SAME STRAIN. ANNA GOLDFEDER.

(Department of Hospitals and New York University, New York, N. Y.)

In previous studies with mouse sarcoma 180 it was found possible to induce resistance in hybrid mice by implants attenuated by specific doses of x-radiation. This paper is concerned with the question whether or not the phenomenon can be applied to tumors of known genetic origin, grown in an inbred strain of animals. Accordingly, an inbred strain of animals and a tumor that originated in it were used in the present experiment. These were obtained from Dr. Halsey J. Bagg, and had the following characteristics: The rats, having been bred by continuous brother and sister mating for more than 15 years, were considered to be homozygous. The tumor, a reticulum cell type lymphosarcoma, originated in this strain of rats and had reached the 75th transplant generation at the start of this experiment. The tumor takes were 100 per cent in young rats, and spontaneous regression very rarely occurred.

Male rats 5 to 6 weeks old were used, with the same technical procedure as in the previous experiments.

Results: The threshold dose for implants of this rat lymphosarcoma lies between 2,200 and 2,500 r in air.

Implants irradiated within these dosages either failed to grow or the tumors produced by them regressed after having reached a certain size. In both cases the rats became resistant to subsequent viable tumor implants.

Seven months have elapsed since the first set of rats was rendered "immune" to the tumor. Tests made recently revealed their persistent resistance to further viable implants.

Thus it is shown that the possibility exists of inducing resistance in an inbred strain of rats to a lymphosarcoma that originated in the same strain.

THE APPEARANCE OF HEPATOMAS FOLLOWING THE INGESTION OF 1,2-BENZANTHRACENE.

FLORENCE R. WHITE, and ALLEN B. ESCHENBRENNER. (National Cancer Institute, Bethesda 14, Md.)

1,2-Benzanthracene is frequently classed as a noncarcinogen, or at most as a compound possessing very weak carcinogenic activity. The literature describes the administration of this hydrocarbon to mice, rats, and rabbits by painting on the skin and by intravenous, subcutaneous, and intraperitoneal injections. Not every mode of administration was used on each species of animals. The only neoplasm reported was one epithelioma following the painting of 30 mice.

The purpose of this communication is to report the occurrence of hepatomas in 2 among 6 rats fed a relatively normal diet to which 0.06 per cent 1,2-benzanthracene had been added. The livers of both showed multiple tumors associated with cirrhosis and resembled those observed after feeding *p*-dimethylaminoazobenzene under similar circumstances.

American Association for Cancer Research, Inc.

36th Annual Meeting

College of Physicians and Surgeons, New York 32, N. Y.
May 8, 1945

Proceedings of Business Sessions

Minutes of the Meeting of the Board of Directors Held May 8, 1945

A meeting of the Board of Directors was held in the Alumni Room on Floor A, College of Physicians and Surgeons, 630 West 168th Street, New York 32, N. Y. The meeting was called by order of the Chairman, Dr. Shields Warren, who requested notices sent to all Directors and dated March 23, 1945 from New Haven, Conn. The meeting was called to order at 1:30 p.m., by the chairman. Directors C. C. Little, J. B. Murphy, A. A. Thibaudeau, Carl Voegtlin, Shields Warren and W. H. Woglom were present.

It was voted that the reading of the minutes of the special meeting of the Board of Directors held October 12, 1944 be waived. Minutes of the meeting had been submitted to the Directors and then were published in *Cancer Research* 1945, Volume 5, page 55.

Report of Officers

The reports of the Chairman and Acting Secretary-Treasurer were read and approved. Dr. A. A. Thibaudeau was nominated and elected Auditor.

Report of Committees

Program Committee.—In the absence of the Chairman, Dr. M. J. Shear, Dr. C. C. Little reported for the Committee. Twenty-seven abstracts were received of papers to have been presented at the Scientific Sessions to have been held in Cleveland but later postponed.

Nominating Committee.—Chairman H. B. Andervont was not present. Dr. A. A. Thibaudeau reported that 24 candidates were nominated for Directors, 8 each for terms of 1, 2, and 3 years as follows:

1 Year

Edmund V. Cowdry
Edward A. Doisy
Francisco Duran-Reynals
John H. Lawrence
John J. Morton
James B. Murphy
Howard C. Taylor, Jr.
Carl Voegtlin

2 Years

Wilton R. Earle
Jacob Furth
Harry S. N. Greene
John G. Kidd
Clarence C. Little
Stanley P. Reiman
Shields Warren
William H. Woglom

3 Years

John J. Bittner
Austin M. Brues
William U. Gardner
Charles B. Huggins
Harold P. Rusch
Murray J. Shear
Leonell C. Strong
George W. Woolley

These names were included on the proxies sent to all members from the office of the Acting Secretary-Treasurer.

Membership Committee.—Chairman W. H. Woglom reported that the Association now had 445 active and seven honorary or emeritus members. Fifty-four members were in active service with the armed forces.

Resignations of the following two members were presented and accepted:

Dick, George F., M.D., University of Chicago, Chicago 37, Ill.
Jobling, James W., M.D., College of Physicians & Surgeons, New York 32, N. Y.

Nominations of 31 applicants to active membership and one to an emeritus membership were presented and they were duly elected members of the Association. They were as follows:

Ball, Zelda B., M.S., University of Minnesota, Minneapolis 14, Minn.
Barnum, Cyrus P., Jr., Ph.D., University of Minnesota, Minneapolis 14, Minn.
Blumenfeld, Charles M., M.D., Western Reserve University, Cleveland 6, Ohio.
Carruthers, Christopher, Ph.D., Barnard Free Skin and Cancer Hospital, Washington and Theresa Avenues, St. Louis 3, Mo.
DeVita, Joseph, V.M.D., 724 Whitney Avenue, New Haven 11, Conn.
Fogg, Lloyd C., Ph.D., Boston University School of Medicine, Boston 18, Mass.
Goldblatt, Harry, M. D., Western Reserve University, 2085 Adelbert Road, Cleveland 6, Ohio.
Goldsmith, Eli D., Ph.D., New York University, Washington Square E, New York 3, N. Y.
Hauser, Harry, M.D., Western Reserve University, 3395 Scranton Road, Cleveland 9, Ohio.
Hesselbach, Marie L., B.A., National Cancer Institute, Bethesda 14, Md.
Jones, Hardin B., Ph.D., University of California, 1106 Oxford Street, Berkeley 4, Calif.
Kurzrok, Raphael, M.D., Morrisania City Hospital, 1016 Fifth Avenue, New York 28, N. Y.
Laipply, Thomas C., M.D., Western Reserve University, 2085 Adelbert Road, Cleveland 6, Ohio.
LeCompte, Philip M., M.D., New England Deaconess Hospital, 195 Pilgrim Road, Boston 15, Mass.

LEUCHTENBERGER, RUDOLF, M.D., Cancer Research Laboratory, Mount Sinai Hospital, 12 East 97th Street, New York 29, N. Y.

LUND, HERBERT, M.D., Western Reserve University, 2085 Adelbert Road, Cleveland 6, Ohio.

MEISSNER, WILLIAM A., M.D., New England Deaconess Hospital, 195 Pilgrim Road, Boston 15, Mass.

MILLER, JAMES A., Ph.D., McArdle Memorial Laboratory, University of Wisconsin, Madison 6, Wis.

OLIVER, CLARENCE P., Ph.D., University of Minnesota, Minneapolis 14, Minn.

RHOADS, ERNEST E., M.D., Western Reserve University, 2085 Adelbert Road, Cleveland 6, Ohio.

RUSSELL, WILLIAM O., M.D., Santa Barbara Cottage Hospital, Santa Barbara, Calif.

SCHENKEN, JOHN R., M.D., Louisiana State University School of Medicine, 1542 Tulane Avenue, New Orleans, La.

SCHNEIDER, WALTER C., Ph.D., McArdle Memorial Laboratory, University of Wisconsin, Madison 6, Wis.

SIEKMANN, CARL F., M.D., State Institute for the Study of Malignant Disease, 663 North Oak Street, Buffalo 3, N. Y.

SILBERBERG, RUTH, M.D., Jewish Hospital, St. Louis, Mo.

SIMPSON, WILLIAM L., Ph.D., Barnard Free Skin and Cancer Hospital, Washington and Theresa Avenues, St. Louis 3, Mo.

SINGHER, HERON O., Ph.D., Memorial Hospital, 444 East 68th Street, New York 21, N. Y.

STRAUS, REUBEN, M.D., Cedars of Lebanon Hospital, 4833 Fountain Avenue, Los Angeles 27, Calif.

VISSCHER, MAURICE B., M.D., University of Minnesota Medical School, Minneapolis 14, Minn.

WHITE, FLORENCE R., Ph.D., National Cancer Institute, Bethesda 14, Md.

ZAMECNIK, PAUL C., M.D., Massachusetts General Hospital, Boston 14, Mass.

EMERITUS MEMBER

SEELIG, M. G., M.D., Barnard Free Skin and Cancer Hospital, Washington and Theresa Avenues, St. Louis 3, Mo.

Journal Committee.—Dr. M. W. S. Schram, Chairman of the Advisory Board of the journal, *Cancer Research,* reported at the request of the Chairman of the Journal Committee, Dr. C. C. Little. *Cancer Research,* the official organ of this Association, during the past year published approximately 680 pages of original articles and 150 pages of abstracts. Among the approximate 700 subscribers to the journal 169 are members of the Association. The journal is supported by grants from three foundations supporting cancer research and by this Association, which has contributed the sum of $500.00 yearly for the past three years. Special credit for the success of the journal was extended to Dr. William H. Woglom, Editor, and to Dr. Clara J. Lynch, Editor of the abstracts section. During the year 23 persons have served on the editorial committee and 49 persons from the United States and England have served as abstractors for the journal.

NEW BUSINESS

It was voted that, "The Secretary extend to Dr. Lawrence A. Pomeroy, Cleveland, Ohio, Chairman of the Local Committee, the appreciation of the Association for his activities in completing the preliminary arrangements for the meeting to have been held in Cleveland."

The count of the proxies for the Directors was reported by the Acting Secretary-Treasurer. The four nominees for directorships for periods of 1, 2, and 3 years receiving the greatest number of votes were as follows:

1 Year	2 Years	3 Years
E. V. COWDRY	J. FURTH	J. J. BITTNER
E. A. DOISY	C. C. LITTLE	W. U. GARDNER
J. B. MURPHY	S. WARREN	C. B. HUGGINS
C. VOEGTLIN	W. H. WOGLOM	M. J. SHEAR

It was voted, "That the Acting Secretary-Treasurer cast one ballot for election of Directors as revealed by the count of the proxies." The ballot was cast and the Directors declared elected.

It was voted that, "The American Association for Cancer Research contribute to the Ewing Memorial Committee the sum of $100.00 to the fund being collected to support graduate instruction and lectureships on the subject of cancer." Dr. Ewing was one of the founders of this Association.

It was voted that, "The Acting Secretary-Treasurer be authorized to submit the proceedings of this meeting for publication in *Cancer Research* and to pay the expenses incurred by such publication."

The meeting adjourned at 2:50 p.m.

SHIELDS WARREN, *Chairman,*
Board of Directors
W. U. GARDNER, *Acting*
Secretary-Treasurer

MINUTES OF THE MEETING OF THE BOARD OF DIRECTORS HELD MAY 8, 1945

The meeting was called at 2:50 p.m. following waiver of previous formal notice of the meeting of the Directors, signed by all Directors present and constituting a quorum for the Directors. Directors Jacob Furth, W. U. Gardner, C. C. Little, J. B. Murphy, M. J. Shear, Carl Voegtlin, Shields Warren, and W. H. Woglom were present. The meeting convened in the Alumni Room, Floor A, College of Physicians and Surgeons, 630 West 168th Street, New York 32, N. Y.

It was voted that, "The reading of the minutes of the previous meeting be dispensed with."

The nominations for the following officers for the ensuing year were then made, namely; Shields Warren, President; W. U. Gardner, Vice-President; and Charles W. Hooker, Acting Secretary-Treasurer.

It was voted, "That the Secretary cast one ballot for the nomination of each of the candidates." The ballots were cast and officers declared nominated.

The meeting adjourned temporarily at 3:10 p.m. for a meeting of the members of the Association.

MINUTES OF THE MEETING OF MEMBERS HELD MAY 8, 1945

A meeting of the members of the Association was held in the Alumni Room, Floor A, College of Physicians and

Surgeons, 630 West 168th Street, New York 32, N. Y. The meeting was called to order at 3:10 p.m. by Dr. Shields Warren, President. Twenty-one members were in attendance.

The minutes of the meeting held March 31, 1942 in Boston, Massachusetts were read and approved.

The nominations for officers in the Association as submitted by the Board of Directors were as follows:

SHIELDS WARREN, *President*
W. U. GARDNER, *Vice-President*
CHARLES W. HOOKER, *Acting Secretary-Treasurer*

It was voted that, "The Secretary be authorized to cast one ballot for the slate of officers as nominated by the Board of Directors." The ballot was so cast and the officers declared duly elected.

It was voted that, "The Board of Directors take immediate action, if considered expedient, or otherwise defer action until the next meeting of the members, to appoint representatives of the Association to the Union of Biological Societies and thereby become affiliated with this group; this representation to be made by two members selected for a period of three years."

The meeting adjourned at 3:40 p.m.

SHIELDS WARREN, *President*
W. U. GARDNER, *Acting Secretary-Treasurer*

Meeting of the Directors reconvened at 3:40 p.m. All the Directors mentioned above were present. In the absence of the newly elected Acting Secretary-Treasurer it was voted that, "W. U. Gardner substitute as Secretary of the meeting of the Directors."

It was voted that, "The President of the Association and Chairman of the Board of Directors be authorized to appoint the members of each of the standing committees." These were as follows:

Program Committee.—M. J. Shear, Chairman; E. V. Cowdry, C. C. Little.

Nominating Committee.—W. U. Gardner, Chairman; J. J. Bittner, Jacob Furth.

Membership Committee.—W. H. Woglom, Chairman; C. B. Huggins, Carl Voegtlin.

Journal Committee.—J. B. Murphy, Chairman; C. C. Little, E. A. Doisy, M. W. S. Schram.

FUTURE MEETING OF THE MEMBERS

It was voted that, "The American Association for Cancer Research plan to hold a meeting of the members during the coming year in conjunction with the meeting of the Federation of the American Societies for Experimental Biology should suspension or modifications of the current rulings of the Office of Defense Transportation permit."

OTHER BUSINESS (GENERAL)

It was unanimously *resolved,* that Charles W. Hooker, Acting Secretary-Treasurer and Shields Warren, President, be and each of them hereby is authorized, in the name and on behalf of the Corporation to open a bank account or bank accounts with such banks, bankers, and/or trust companies as they or each of them shall determine, and to deposit therein to the credit of the Corporation from time to time any and all monies and checks of the Corporation; and

Resolved, that the banks, bankers, and/or trust companies so designated as depositaries of the Corporation be, and they hereby are, severally authorized to honor and pay all checks, drafts, and other orders for the payment of money drawn upon such account or accounts (including checks, drafts, or other orders of one or both of the persons making, signing, or drawing them) made, signed or drawn by the following persons: Charles W. Hooker or Shields Warren.

It was voted that, "The sum of $150.00 be made available to the Acting Secretary-Treasurer for the procurement of secretarial assistance."

It was voted that, "Because of the temporary addresses of so many members in the armed forces the publication of the By-Laws, List of Officers and Members of the Association should be deferred for one more year." In the past it has been the policy of this Association to provide such publication every other year.

It was voted that, "Members of the Association in active service with the armed forces continue to be exempt from dues during the period of such service."

Dr. Carl Voegtlin was appointed Auditor for the Association.

COMMENDATION OF AMERICAN CANCER SOCIETY

It was voted that, "In view of the important action of the American Cancer Society which has initiated the first comprehensive approach to nationwide support of cancer research, this Association endorse the objectives of the American Cancer Society, offer cooperation, and express the thanks of the research workers who are our members to that society for the breadth of vision manifest in its program."

INTERNATIONAL CANCER RESEARCH CONGRESS

The following motion was made and unanimously voted: "The President is hereby authorized to appoint a small committee to act as a liaison with foreign cancer investigators in occupied and neutral countries, and foreign institutions in occupied and neutral countries engaged or interested in conducting cancer investigations in those countries with a view to offering them encouragement and help in reestablishing cancer research abroad and prepare the foundation for a future International Cancer Research Congress." Shields Warren appointed the following committee: M. J. Shear, Chairman; Robert Chambers, and Carl Voegtlin.

ADDITIONAL COMMITTEES

The question of the desirability of a committee on conferences to be made up of representatives from the several

foundations supporting cancer research and of the several national groups interested in cancer research was discussed. Also the desirability of a committee to study and report to the membership of the Association the various facilities provided by foundations, governmental agencies, etc., in the support of cancer research was discussed. The suggestion was made that, should the officers of the Association consider such committees desirable, they be temporarily appointed and considered at future meetings of the board of directors.

The meeting adjourned at 5:00 p.m.

SHIELDS WARREN, *President*
W. U. GARDNER, *Acting*
Secretary-Treasurer

Abstracts

Reports of Research

Potential Differences in the Skin of Mice during Carcinogenesis. BURROWS, H., ROE, E. M. F., and SCHOBER, B. [Chester Beatty Research Inst., Roy. Cancer Hosp. (Free), London, England] *Cancer Research,* **5**:524-538. 1945.

Potential differences (p.d.) were measured, by a vacuum tube microvoltmeter, between positions on the surface of tumor tissue and neighboring normal tissue in the skin of MRC mice (from the Medical Research Council), the tumors being produced by painting with the carcinogen 9,10-dimethyl-1,2-benzanthracene in acetone. P.d. changes during carcinogenesis were compared with similar measurements in control mice painted with acetone.

In individual animals a statistical analysis of measurements showed only one significant result, *i.e.,* at "clinical" stages distinguished during carcinogenesis, p.d's tended to remain at a given level, although there was diversity about this level at certain other stages.

For all animals taken together it was found that there was no change in p.d. due to painting with acetone or with carcinogen in acetone. The mean p.d.'s in the animals before painting or in those painted with acetone alone showed an electrical asymmetry (left side of animal positive) that increased with age, as did the variance and standard deviation of these p.d.'s. The animals painted with carcinogen in acetone showed a maximum variance of the p.d. during the hypertrophic stage, and a smaller variance of p.d. during the malignant stage, than during the rest of the experiment. There was also some evidence that the mean p.d. is more negative (with reference to normal skin) in the period immediately preceding and during the early stages of development of a papilloma than during the rest of the experiment, and there was a greater variance and a change in the level of the p.d. during this period. There was no such change in the mean p.d. or in the variance in the period immediately preceding and during the early stages of malignancy, although there was a definite change in the level of p.d. in this period.—Authors' abstract.

Further Studies on the Relation between Radiation Effects, Cell Viability, and Induced Resistance to Malignant Growth. II. Effects of Roentgen Rays on Bagg-Jacksen Mouse Carcinoma 755 Irradiated in Vitro and in Situ. GOLDFEDER, A. [New York Univ. Med. Coll., and Bellevue Hosp., New York, N. Y.] *Radiology,* **44**:283-292. 1945.

The tumor used in this experiment was the Bagg-Jacksen mammary mouse cancer 755, grown in an inbred strain of mice, C57"Y" black. X-radiation was applied to the tumor fragments *in vitro* and *in situ*. With a dose of 3,500 r in air at high voltage (h.v.l. 0.9 mm. Cu) *in vitro* there were 58.6% "takes," while with 4,500 r growth of the irradiated implants was prevented. The dose required to destroy tumor *in situ* does not appear to differ greatly from that necessary to prevent an implant from producing a tumor when an inbred strain of mice is used.—R. E. S.

The Unsaponifiable Residue of Human Liver. I. Preparation and Primary Chromatographic Fractionation. STANGER, D. W., STEINER, P. E., and BOLYARD, M. N. [Univ. of Chicago, Chicago, Ill.] *J. Am. Chem. Soc.,* **66**:1621-1623. 1944.

The cholesterol-free residues of the unsaponifiable fraction from livers of persons dying of cancer and of non-neoplastic disease, respectively, have been separated into 5 fractions by chromatographic adsorption, and slight differences have been found in the percentage composition of the cancer and non-cancer residues. (The "cancer livers" themselves contained no tumor tissue, or were only slightly involved.) One fraction was found to be mainly saturated hydrocarbon, and another fraction was considered to be largely hepene. Further investigation of the residues is reported to be in progress.—H. J. C.

Potassium and Calcium in Epidermal Carcinogenesis Induced by Methylcholanthrene. SUNTZEFF, V., and CARRUTHERS, C. [Barnard Free Skin and Cancer Hosp., and Washington Univ. Sch. of Med., St. Louis, Mo.] *J. Biol. Chem.,* **153**:521-527. 1944.

In these studies a transplantable squamous cell carcinoma was employed that was originally produced in the epidermis of a Swiss mouse by the application of methylcholanthrene. This tissue was found to contain less calcium than hyperplastic epidermis, which in turn contained less calcium than normal epidermis; the potassium content of the carcinoma likewise was less than that of hyperplastic and normal epidermis. The precautions necessary in the selection of tissue are noted.—H. J. C.

Chemical Studies on the Transformation of Mouse Epidermis by Methylcholanthrene to Squamous Cell Carcinoma. CARRUTHERS, C., and SUNTZEFF, V. [Barnard Free Skin and Cancer Hosp., and Washington Univ. Sch. of Med., St. Louis, Mo.] *J. Biol. Chem.,* **155**:459-464. 1944.

A single application of methylcholanthrene to mouse epidermis produced a rapid and extensive decrease in the calcium and iron content of the tissue. Continued treatments had little effect on the calcium content but did cause a further decrease in the iron content. It was found that the amounts of potassium, sodium, magnesium, and ascorbic acid were not decreased significantly by single or repeated methylcholanthrene applications. The transplantable skin carcinoma resulting from methylcholanthrene, when it was small, solid, and practically free of necrotic tissue, contained less of all the minerals than did

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normal and hyperplastic epidermis. However, necrotic tumor tissue had more calcium and sodium, and less potassium and magnesium, than did the small, solid tumors.—H. J. C.

The Desoxyribonucleic Acid Content of Isolated Nuclei of Tumor Cells. Dounce, A. L. [Sch. of Med. and Dentistry, Univ. of Rochester, Rochester, N. Y.] *J. Biol. Chem.*, **151**:235-240. 1943.

The results presented in this paper indicate that the theory that "the fundamental cause of increased division rate and malignancy is the excess amount of nucleic acid in the tumor" may be unsound. It was observed that the nuclei of Walker carcinosarcoma 256 in the resting stage have nearly the same concentration of desoxyribonucleic acid as the nuclei of cells of normal rat liver in the resting stage and that the nuclei of hepatoma 31 have a lower content of the acid. Nuclei from bird erythrocytes and fish spermatozoa have a higher content of desoxynucleic acid than the nuclei of the tumors that were studied.—H. J. C.

Nucleic Acids of Rat Liver and Hepatoma: Their Metabolic Turnover in Relation to Growth. Brues, A. M., Tracy, M. M., and Cohn, W. E. [Med. Labs. of Collis P. Huntington Memorial Hosp. of Harvard Univ. at Massachusetts Gen. Hosp., Boston, Mass.] *J. Biol. Chem.*, **155**:619-633. 1944; *Publication No. 593 of the Cancer Commission of Harvard Univ.*

Thymonucleic acid from hepatoma 31 shows a phosphorus and a nitrogen content equal to that of thymonucleic acid from normal tissues. The turnover of thymonucleic acid phosphorus, as measured by the uptake of radioactive phosphorus, is very slow in non-growing liver, intermediate in hepatoma 31, and rapid in regenerating liver. The implications of thymonucleic acid metabolism with regard to cell growth and nuclear stability are discussed.—H. J. C.

The Cytochrome C Content of Normal and Neoplastic Mammalian Epithelium and Its Correlation with Body Mass. Rosenthal, O., and Drabkin, D. L. [Sch. of Med., Univ. of Pennsylvania, Philadelphia, Pa.] *J. Biol. Chem.*, **150**:131-141. 1943.

A deficiency of cytochrome *c* cannot be regarded as exclusively characteristic of malignant neoplasms because certain tissues (of the rat and of man) subject to spontaneous neoplasms were found not to differ significantly in cytochrome *c* content from the neoplasms derived from these tissues. It was found that the concentration of cytochrome *c* in the same epithelial tissues from different species in many cases was approximately an inverse function of body mass. In man, all the normal epithelial tissues studied were found to be low in cytochrome *c*.—H. J. C.

Steroid Excretion in a Case of Adrenocortical Carcinoma. I. The Isolation of Δ^5-Androstenetriol-3(β),16,17. Hirschmann, H. [Univ. of Pennsylvania, Philadelphia, Pa., and Sch. of Med., West. Reserve Univ., and Lakeside Hosp., Cleveland, Ohio] *J. Biol. Chem.*, **150**:363-379. 1943.

From the urine of a boy with an adrenocortical carcinoma there was isolated a new steroid, which was identified as Δ^5-androstenetriol-3(β),16,17.—H. J. C.

Steroid Excretion in a Case of Adrenocortical Carcinoma. II. An Examination of the Non-Ketonic Fraction Precipitable with Digitonin. Hirschmann, H., and Hirschmann, F. B. [Sch. of Med., West. Reserve Univ., and Lakeside Hosp., Cleveland, Ohio] *J. Biol. Chem.*, **157**:601-612. 1945.

Crystalline acetates of Δ^5-pregnenediol-3(β),20(α), Δ^5-androstenediol-3(β),17(α), and 3 unidentified compounds have been obtained from the urine of a boy with adrenocortical carcinoma. The parent substances of these acetates have not been encountered previously in human urine. Pregnenediol and androstenediol may be derived at least in part from substances formed in the adrenal tumor.—H. J. C.

Isolation of a Urinary Steroid with an Oxygen Atom at Carbon 11. Mason, H. L. [Mayo Foundation, Rochester, Minn.] *J. Biol. Chem.*, **158**:719-720. 1945.

A compound with an oxygen atom at C 11 of the steroid nucleus has been isolated from the urine of 7 patients with tumors or hyperplasia of the adrenal cortex. It is suggested that the compound is androstane-3α,11β-diol-17-one.—H. J. C.

Metabolism of the Steroid Hormones. The Excretion of Estrogenic Material by Ovariectomized Mice Bearing Adrenal Tumors. Doreman, R. I., and Gardner, W. U. [Brush Foundation, West. Reserve Univ. Sch. of Med., and Lakeside Hosp., Cleveland, Ohio, and Yale Univ. Sch. of Med., New Haven, Conn.] *Endocrinology*, **34**:421-423. 1944.

The concentration of urinary and fecal estrogens was studied in 10 ovariectomized mice of the NH strain bearing spontaneous adrenal tumors and compared to the estrogen excretion of 11 intact normal females of the same strain. The tumor-bearing ovariectomized mice excreted 0.19 I.U. daily in the urine and 0.10 I.U. in the feces, while the normal females excreted 0.03 I.U. in urine and 0.04 I.U. in the feces. Thus the tumor-bearing mice excreted about 4 times as much estrogenic material as the intact normal females.—C. A. P.

Estrogen Production by Sertoli Cell Tumors of the Testis. Huggins, C., and Moulder, P. V. [Univ. of Chicago, Chicago, Ill.] *Cancer Research*, **5**:510-514. 1945.

Feminizing tumors of the testis of dogs are rich in lipids and estrogen. They are growths of the sustentacular cells of Sertoli; these cells in the testicular tubule produce estrogen.—Authors' abstract.

Hepatic "Inactivation" of Estrogens. Cantarow, A., Paschkis, K. E., and Rakoff, A. E. [Jefferson Med. Coll., Philadelphia, Pa.] *Science*, **101**:558. 1945.

Lipschütz and his co-workers reported recently that estriol and equilinin, when absorbed from pellets implanted in the spleen, had relatively little fibromatogenic effect. This result indicated to them that the hormones were inactivated in the liver. However, Cantarow and his associates think that this conclusion is unwarranted, since estrogens were previously shown by them to be excreted in the bile and thus may be removed from the circulation without being activated in the liver.—R. B.

Induction and Prevention of Fibromyoepithelioma of the Utricular Bed in Male Guinea Pigs. LIPSCHÜTZ, A., YANINE, D., SCHWARZ, J., BRUZZONE, S., ACUÑA, J., and SILBERMAN, S. [Nat. Health Service, Santiago, Chile] *Cancer Research,* **5**:515-523. 1945.

Tumorous growths attaining in some cases a diameter of 2 cm. or more were elicited in the prostatic region of male guinea pigs by the prolonged action of various estrogens (α-estradiol, estradiol dipropionate, stilbestrol, and hexestrol) absorbed from subcutaneously implanted tablets. The earliest tumor appeared 108 days after their implantation. The incidence increased greatly with the greater length of estrogenic action. In experiments lasting from 7 to 11 months the incidence reached more than 40% in castrated animals and seemingly less in intact ones.

The tumors were fibromyoepitheliomas, and resulted from further hyperplastic growth of the fibromuscular pre-utricular tissue and metaplastic growth of the mucosa of the utriculus and a limited area of the vasa deferentia. The fibromyomatous part of the pre-utricular tumor was structurally different from the estrogen-induced abdominal fibroid of peritoneal origin. Whereas fibroblasts originating from the peritoneum prevailed in the latter, bundles of smooth muscle fibers originating from the utricular bed prevailed in the former. The proliferative, and especially the metaplastic, changes of the utricular mucosa started not at its extreme caudal end, but a certain distance cephalad.

The estrogen-induced fibromyoepitheliomas of the utricular bed were prevented when, after a few weeks of action of the subcutaneously implanted estrogenic tablets, tablets of antifibromatogenic steroids, such as progesterone, desoxycorticosterone acetate, and testosterone propionate, were implanted. However, when the antifibromatogenic steroids were added 5 to 6 months after the implantation of the estrogenic tablet and allowed to act for another 4 months, fibromyoepitheliomas of the utricular bed were present in a number of cases, and their structure was the same as that of tumors arising from estrogen alone. But the incidence of these tumors was lower and their size smaller than after estrogens only.

The question is discussed whether there are quantitative differences of response to antifibromatogenic steroids between the two types of estrogen-induced mesodermal tumors, *i.e.*, peritoneal fibroid and the pre-utricular fibromyoma.—Authors' summary.

The Influence of 11-Dehydro-17-Hydroxycorticosterone (Compound E) on the Growth of a Malignant Tumor in the Mouse. HEILMAN, F. R., and KENDALL, E. C. [Mayo Foundation, Rochester, Minn.] *Endocrinology,* **34**:416-420. 1944.

A transplanted lymphosarcoma that takes (practically 100%) in 10 to 14 days and kills within 14 to 18 days after the tumor is palpable was treated with compound E. Mice that received 0.3 mgm. of compound E in the drinking water daily failed to grow the tumor during 23 days of treatment and for 13 days afterward. However, following this period, 4 of 7 mice began to grow the tumor. Well developed tumors also underwent a rapid regression when the mice received compound E in the drinking water. Injection of 1 to 2 mgm. of compound E caused regression of the tumors in varying degrees; regression was faster and more consistent in females than in males. Castration of male mice or treatment with estradiol propionate appeared to increase the effect of compound E. In both sexes the effect of compound E was enhanced by simultaneous administration of phlorhizin. The influence of compound E appeared to depend on stimulation of the rate of the catabolism of proteins to a degree that resulted in the death of the malignant cells. The normal tissue cells of the animal, although perhaps placed under severe strain, did not appear to undergo permanent injury.

Although dramatic and apparently complete cures were produced, they were only temporary in a majority of the animals. After an interval of a few days or weeks the tumors usually recurred, and this time they were completely refractory to treatment, and in every case eventually caused the death of the animal. The refractory state was apparently inherent in the tumor cell and did not depend on the host.—C. A. P.

Some Influences of Hormones on the Growth and Persistence of Transplanted Testicular Tumors. GARDNER, W. U. [Yale Univ. Sch. of Med., New Haven, Conn.] *Cancer Research,* **5**:497-505. 1945.

Two testicular interstitial cell tumors, one that arose in a mouse of the A strain that had received triphenylethylene and one that arose in a hybrid mouse (A♀× C3H♂) that had received stilbestrol, were transplanted subcutaneously into other mice for 9 and 4 generations respectively. The tumors consisted predominantly of cells of "generation two," as described previously.

The tumors failed to grow in all 34 genetically susceptible and untreated mice into which they were implanted. Seventeen mice of the A strain received transplants, and no growth occurred during the subsequent 51 to 204 days. Estrogens given at this time resulted in the growth of 16 of the "tumor rests." The rests grew as rapidly as recently transplanted tumors. The transplanted tumors that developed in estrogen-treated mice continued to grow, or persisted, when treatment was discontinued for periods of 14 to 188 days. The tumors persisted for 10 to 28 days in hypophysectomized mice although the hosts lost weight. The rate of growth and the structure of the tumors did not change appreciably during a period of 3 years of successive transplantation. The testicular tumors failed to grow in estrogen-treated mice of unrelated strains.—Author's summary.

The Physiology of the Prostate Gland. HUGGINS, C. [Univ. of Chicago, Chicago, Ill.] *Physiol. Rev.,* **25**:281-295. 1945.

This article is devoted principally to the physiology of the normal prostate in man and the lower animals. A short section on benign prostatic hypertrophy describes the salient features of this condition. The main part of the evidence indicates that the hypertrophy is due in part to androgen stimulation even though androgenic hormones are produced in smaller amounts in older men, in whom the condition is common, than in young men, in whom it is rare. To account for this set of facts it is suggested that the threshold for stimulation by androgen is lowered in the part of the prostate becoming hypertrophic, so

that it is still stimulated to grow by the reduced amount of hormone.—R. B.

The Preparation of Concentrates of Specific Substances from Urine and Feces in Leucemia. Turner, D. L., and Miller, F. R. [Charlotte Drake Cardeza Foundation, Jefferson Med. Coll., Philadelphia, Pa.] *J. Biol. Chem.*, **147**: 573-579. 1943.

A method is described for the concentration of biologically active materials from the urine and feces of patients with leukemia and related diseases. The concentrates produce specific myeloid or lymphoid proliferation in guinea pigs. The factors responsible for the two types of hyperplasia are interconvertible by oxidation or reduction involving hydroxyl and carbonyl groups respectively.—H. J. C.

A Characterization of a Urinary Fraction Capable of Producing Myeloid Metaplasia in Guinea Pigs. Hirschmann, H., Heinle, R. W., and Wearn, J. T. [Sch. of Med., West. Reserve Univ., and Lakeside Hosp., Cleveland, Ohio] *Proc. Soc. Exper. Biol. & Med.*, **58**:5-6. 1945.

Myeloid hyperplasia and metaplasia in guinea pigs have been reported as following the injection of extracts and adsorbates of human urine obtained from patients with chronic myeloid leukemia. The present report deals with some chemical and physical properties of a further purified fraction.

The benzoic acid adsorbate was extracted with acetone, and the insoluble residue leached with sufficient *M*/2 boric acid buffer (pH 8.5) to produce an extract of pH greater than 7. The solution was acidified with acetic acid (pH 4.3) and treated with 1.25 volumes of acetone in the cold. The resulting precipitate was dissolved in saline solution containing phosphate buffer (final pH 7.5). Assay of this material in guinea pigs gave an adequate biological response for testing its stability.

Activity was partly lost after heating on the steam bath for 1 hour at pH 7.5 or after exposure to a solution of pH 1.1 at 27.5° C. for 24 hours. Most of the activity was lost in a solution of pH 11.2 at 27.5° within 24 hours. Potency was retained after dialysis against distilled water through a Visking membrane (66 hours at 3°). The preparation contained nitrogen (mainly biuret-nitrogen), a trace of phosphorus, and a considerable amount of carbohydrate. Treatment with trypsin produced an increase of nitrogen not precipitable with trichloracetic acid and a loss of about 4/5 of the biological activity.

The data suggest that the active principle is a protein or glycoprotein.—M. B.

Tumor of Rat Testis Produced by Heterotransplantation of Infantile Testis to Spleen of Adult Castrate. Biskind, M. S., and Biskind, G. R. [Beth Israel Hosp., New York, N. Y., and Mt. Zion Hosp., and Univ. of California Med. Sch., San Francisco, Calif.] *Proc. Soc. Exper. Biol. & Med.*, **59**:4-8. 1945.

Adult rats of the Sherman strain were castrated, and a testis from an infantile rat of the same strain was implanted into the spleen of each adult. Similar implantation was also made into the spleens of noncastrated animals. The animals were killed at intervals of from 8 to 11 months after the implantation. In 2 intact animals the implants were found at autopsy to have increased about tenfold in volume. In both cases there was extensive degeneration of the tubules and little remaining interstitial tissue. Numerous large cystic areas were present. In castrated animals, the implants had increased in volume from 50 to more than 100 times. These tumors bore a striking resemblance to the granulosa-cell tumors of the ovary produced by transplanting one ovary to the spleen and removing the other. The cellular components apparently derived from the tubules and the interstitial cell mass in the testicular tumor are indistinguishable from the granulosa-cell and theca-cell components, respectively, of the ovarian tumor. Also, portions of the testicular tumor were reminiscent of the structure seen in arrhenoblastoma.—M. B.

The Production of Carcinoma and Sarcoma in Transplanted Embryonic Tissues. Greene, H. S. N. [Yale Univ. Sch. of Med., New Haven, Conn.] *Science*, **101**: 644-645. 1945.

Embryonic mouse tissues, implanted together with crystalline methylcholanthrene in various sites in adult mice, manifested cellular and structural changes characteristic of cancer within 35 days, whereas 90 to 200 days are required before comparable changes appear in adult tissues. Various embryonic organs and tissues including lung, stomach, intestine, skin, muscle, and cartilage gave a variety of tumors that spread to the tissues of the host and metastasized, and that were in most cases clearly derived from the implants. Colloid carcinoma or fundic adenocarcinoma was produced in the stomach of the host by the application of chemical carcinogens to embryonic transplants. In the subcutaneous regions, both carcinomas and sarcomas developed. The results appeared to be the same whether the adult hosts were of the same strain as, or of a different strain from, that providing the embryonic tissue.

Tansplanted embryonic guinea pig tissues were found to be as susceptible to carcinogenesis as the embryonic mouse tissues, although adult guinea pigs are relatively resistant to the action of carcinogenic chemicals.—R. B.

Liver Changes Associated with a Transplantable Granulosa Cell Carcinoma in Mice. Furth, J., and Boon, M. C. [Cornell Univ. Med. Coll., and New York Hosp., New York, N. Y.] *Proc. Soc. Exper. Biol. & Med.*, **58**:112-114. 1945.

Granulosa-cell tumors were induced in mice with x-ray by a single dose of 175 or 350 r. In one completed series of 78 mice living 10 months or more, ovarian tumors appeared in 49. Four of the induced tumors metastasized to distant organs. Two of the 4 were successfully transplanted subcutaneously or intraperitoneally into related mice. Another similar tumor, which did not metastasize, also proved transplantable. Liver changes occurred in most mice that carried the transplanted tumor for several weeks. The changes consisted in a cavernous dilatation of liver sinusoids, leading to thrombosis and scar formation. The relation of sex hormones to liver changes is discussed.—M. B.

The Incidence of Adrenal Cortical Carcinoma in Male Mice of the Extreme Dilution Strain over One Year of Age. WOOLLEY, G. W., and LITTLE, C. C. [Roscoe B. Jackson Memorial Lab., Bar Harbor, Me.] *Cancer Research,* **5**:506-509. 1945.

Two groups of strain ce mice were studied. The groups consisted of: (a) 23 castrated male mice and (b) 16 intact male mice. Castration was performed when the mice were 1 to 3 days of age. The animals were autopsied when they were from 13 to 29 months of age. Adrenal cortical carcinomas were found in 100% of the castrate mice and in none of the intact mice. The condition of the accessory sex organs of the castrate mice indicated that they were being subjected to influences similar to sex hormones, that these were multiple, and that the amount and nature of these varied from individual to individual.—Authors' abstract.

Spontaneous Transmissible Tumours in the Syrian Hamster. ASHBEL, R. [Hebrew Univ., Jerusalem, Palestine] *Nature,* **155**:607. 1945.

Thirteen spontaneous tumors occurred during 7 years among 1,000 Syrian hamsters descended from a single family. These tumors were: (1) a polymorph sarcoma transmissible by graft or by heart blood but not by filtrate; (2) a polymorph sarcoma transmissible by graft; (3) a carcinoma "embedded in pancreatic tissue," graftable; (4) cortical hypernephromas in the adrenals in 10 hamsters (sex not stated), only 2 transmissible by subcutaneous or intraperitoneal graft.—E. L. K.

The Genetic Aspects of the Enzyme-Virus Theory of Cancer. POTTER, V. R. [Univ. of Wisconsin Med. Sch., Madison, Wis.] *Science,* **101**:609-610. 1945.

A short discussion, with bibliography, of recent advances in cytology, virology, enzymology, and genetics, as they influence our ideas of the nature of cancer, with special reference to the author's enzyme-virus theory of cancer (*Cancer Research,* **3**:358. 1943; abstr. in **3**:490. 1943).—R. B.

CONFERENCE ON GASTRIC CANCER

This conference was held Nov. 11, 1944, at Memorial Hospital, New York, N. Y., under the auspices of the Committee on Cooperation in Cancer Research, National Advisory Cancer Council, U. S. Public Health Service. Abstracts of the papers follow:

Gastric Physiology in Relation to Gastric Cancer. IVY, A. C. [Northwest. Univ. Med. Sch., Chicago, Ill.] *J. Nat. Cancer Inst.,* **5**:313-330. 1945.

A general discussion of certain aspects of gastric physiology and their possible relationship to gastric carcinoma. Special emphasis is given to: (1) mucus secretion, its physiology and protective significance; (2) chronic gastritis, its occurrence and relationship to malignant disease; and (3) achlorhydria and pernicious anemia, their relation to atrophic enteritis and carcinoma. There is a comprehensive review of pertinent literature, with critical comments, showing the present state of our knowledge of these subjects and pointing out lines of future investigation. The bibliography contains 103 references.—R. A. H.

Genetic Analysis of the Induction of Tumors by Methylcholanthrene. IX. Induced and Spontaneous Adenocarcinomas of the Stomach in Mice. STRONG, L. C. [Yale Univ. Sch. of Med., New Haven, Conn.] *J. Nat. Cancer Inst.,* **5**:339-356. 1945.

By tandem crossing of 3 inbred strains of mice a heterozygous population was obtained. For 5 generations every individual was given a subcutaneous injection of methylcholanthrene, and breeding with selection away from local tumors was carried out. This produced sublines of mice that developed a great variety of neoplastic lesions. These lines have now been continued by brother to sister inbreeding for 9 to 11 generations without further treatment with carcinogen. Observations on gastric neoplasms in one of these sublines are presented in this discussion.

As the degree of homozygosity has increased, the incidence of spontaneous glandular neoplasms of the stomach has also increased, until, of the last group observed, 53% of the animals autopsied when 551 to 600 days of age showed these lesions. Three types have been noted (1) adenocarcinomas, 80.5% of lesions, (2) adenomas, 8.2%, and (3) adenomatous hyperplasias, 11.2%. The adenocarcinomas are histologically malignant, metastasize to regional lymph nodes, and invade contiguous tissues. Animals with these adenocarcinomas were found to be hypochlorhydric and moderately anemic, as compared to normal mice of the same age.

Preliminary experimentation indicates that the main gene determining the tendency for gastric carcinoma development is dominant and carried in the chromosome for brown coat color. The author advances the theory that the injection of methylcholanthrene in the original heterozygous population caused: (1) a somatic mutation of the gastric mucosa giving rise to gastric carcinoma in the treated animals, and (2) a hereditary germinal mutation increasing the susceptibility of subsequent untreated generations to spontaneous gastric neoplasms.—R. A. H.

Superficial Spreading Type of Carcinoma of the Stomach. STOUT, A. P. [Coll. of Physicians and Surgeons, Columbia Univ., and Presbyterian Hosp., New York, N. Y.] *J. Nat. Cancer Inst.,* **5**:363. 1945.

A study of 23 cases of superficial spreading carcinoma of the stomach indicates that this type of carcinoma metastasizes to the regional nodes less frequently than do other types and, therefore, appears to be more amenable to resection. Six of the patients under observation have already passed the fifth postoperative year, and 11 others are still alive less than 5 years after operation.—R. A. H.

Radical Surgery for Gastric Cancer. BRUNSCHWIG, A. [Univ. of Chicago, Chicago, Ill.] *J. Nat. Cancer Inst.,* **5**:365. 1945.

The author advocates massive resection of the stomach and contiguous organs for palliation in cases of far advanced carcinoma of the stomach. Three cases are reviewed. In all, 8 such massive resections, with 2 postoperative deaths, have been performed by the author.—R. A. H.

Physiology of Mucus Secretion. HOLLANDER, F. [Mt. Sinai Hosp., New York, N. Y.] *J. Nat. Cancer Inst.,* **5**:367. 1945.

Gastric mucus secretion was stimulated by mild chemical

irritants; the pH and histology of the secretion were studied. Several stimuli including 50 to 85% ethyl alcohol and a saturated aqueous solution of ether, applied to the mucosa of gastric corpus pouches not secreting acid at the time, called forth a secretion the pH values of which varied widely but clustered about pH 7. This was interpreted as indicating the presence of transudate and inflammatory exudate, as well as mucus, in the specimens. Clove oil, on the other hand, gave a secretion with a narrow pH range and a clearly defined peak at pH 8.4. The buffer capacity of the mucus, between the initial pH and 3.5, ranged as high as 84 m*N* with the chloride concentration between 57 and 186 m*N*, neither of these characteristics being correlated with the pH value. The mucus secreted contained large amounts of columnar cells. The significance of the observations in relation to gastritis and cancer is discussed.—R. A. H.

Excretion of Sulfa Drugs by the Stomachs of Normal Persons and Patients with Gastritis, Peptic Ulcer, and Gastric Cancer. Schiff, L. [Univ. of Cincinnati Med. Sch., Cincinnati, Ohio] *J. Nat. Cancer Inst.*, **5**: 369-370. 1945.

Sodium salts of sulfadiazine and sulfapyridine were given intravenously to human subjects, and the rate of excretion into the gastric juice was studied. The ratio of the concentration of sulfadiazine in the gastric juice to that in the blood was usually 0.45 or less for normal persons, and for patients with atrophic gastritis, peptic ulcer, and less extensive gastric cancer, while for 7 patients with extensive gastric carcinoma the mean ratio was 0.69. No correlation could be demonstrated between this concentration ratio and free or total acidity or pH of the gastric contents. In the case of sulfapyridine, however, the concentration ratio was usually between 4 and 8 in the normal pH range, but was found to approach 1 as the pH of the gastric contents approached 7 to 8. By postulating a simple diffusion mechanism for the gastric secretion of sulfonamides, the concentration ratios could be predicted with accuracy from the dissociation constant of the sulfonamide administered.—R. A. H.

Disturbances of Sugar Metabolism in Gastric Cancer. Singher, H. O. [Memorial Hosp., New York, N. Y.] *J. Nat. Cancer Inst.*, **5**:371-373. 1945.

In order to study the abnormal carbohydrate metabolism of patients with gastric cancer under more controlled conditions, subjects were maintained for 3 to 4 weeks on an experimental diet, and intravenous, as well as oral, glucose tolerance curves were studied. Under these conditions the curves showed the aberrations that have been described as typical for patients with gastric cancer, *i.e.*, a somewhat elevated fasting and maximum blood glucose concentration with a noticeably slow return to the fasting level. To determine if this slow disappearance of glucose from the blood was due to a decrease or loss in the effectiveness of insulin, 0.1 unit of insulin per kgm. body weight was given intravenously to normal subjects and to patients with gastric cancer. As this produced the same drop in blood sugar levels in the two groups, it was concluded that the presence of cancer does not affect the insulin hypoglycemic reaction.

The relation of adrenal cortical hormone to carbohydrate metabolism was then investigated. The fasting liver glycogen content was determined in 27 patients with gastrointestinal cancer and in 9 with nonneoplastic gastrointestinal disorders. The results demonstrated no differences in the two groups. Liver glycogen was then determined in similar groups of subjects, after the administration of large doses of glucose. The results suggested that patients with gastrointestinal cancer have an impaired ability to form or store glycogen in the liver. When adrenal cortical extracts were given along with the glucose, evidence was obtained suggesting that the adrenal hormone promotes glycogen storage in the liver under these conditions.

Although a good deal of confirmatory evidence must still be obtained, these data indicate that the liver-adrenal cortex mechanism is a factor in the disturbed carbohydrate metabolism of patients with gastrointestinal cancer.—R. A. H.

Multiple Cancers of the Human Gastrointestinal Tract. Warren, S. [Harvard Med. Sch., Boston, Mass.] *J. Nat. Cancer Inst.*, **5**:375. 1945.

Multiple cancers occur in about 6% of cancer cases, according to necropsy observations in 3,974 cases of malignant disease. "This is tenfold or elevenfold the expected incidence and may be assumed to represent a susceptibility to cancer in certain persons." Nine per cent of persons with gastric cancer were found to have multiple malignant growths; in no case were the multiple lesions confined to the stomach. Fifteen per cent of persons with cancer of the large intestine had multiple malignant lesions; this higher figure illustrates the effect of precancerous multiple polyposis, which is known to be more frequent in the large intestine than in the stomach. In 29% of the cases of multiple cancer of the large intestine, the lesions were restricted to that organ.—R. A. H.

Observations on Chronic Gastritis and Cancer. Meissner, W. A. [New England Deaconess Hosp., Boston, Mass.] *J. Nat. Cancer Inst.*, **5**:377. 1945.

From a histological study of 200 stomachs surgically removed it was concluded that the average severity of chronic gastritis, if present, is no greater in patients with gastric cancer than in those with gastric or duodenal ulcer.—R. A. H.

Discussions were held after the first and second papers and at the conclusion of the conference. The original should be consulted for comments on such topics as the significance of cell desquamation during mucus secretion, intestinal metaplasia of gastric mucosa in relation to gastric neoplasia, the negative results in attempts to induce tumors of the glandular stomach in mice, the possibility that selection (without mutation) could account for the development of the "stomach cancer" strain of mice, and hepatic dysfunction in cases of cancer in human subjects.—R. A. H.

Clinical and Pathological Reports

Clinical investigations are sometimes included under Reports of Research

Radiation

Roentgen Therapy of Mammary Carcinoma: Survival Study Based on 731 Cases. Gratzek, F. R., and Stenstrom, K. W. [Univ. of Minnesota, and Univ. Hosps., Minneapolis, Minn.] *Radiology,* **44**:44-57. 1945.

This study is based on 731 cases of mammary carcinoma in all stages of the disease. In the group of 254 patients with primary operable lesions, who received surgery and postoperative irradiation, the 5 year survival rate was 51%. The over-all survival rate for all stages treated by irradiation alone or in combination with surgery was 27%. Prognosis in bilateral carcinoma is very poor. The changes in technic of treatment and dosage during the past 20 years are described. Most women in whom mammary carcinoma has occurred are urged to have permanent sterilization. It appears that early diagnosis and immediate irradiation following radical mastectomy give the best results.—R. E. S.

Principles of Treatment of Carcinoma Cervix Uteri by Radiotherapy. Sandler, B. [Roy. Cancer Hosp. (Free), London, England] *Proc. Roy. Soc. Med.,* **38**:175-183. 1945.

The paper is a review of the present position of the problem indicated in the title. After surveying the biological effects of the radiation, with particular emphasis on the production of chromosome breaks by ionizing radiations, the author discusses the more physical aspects, particularly the way in which the dose is built up from several sources of radiation. It is insisted that the radium problem must be treated as a three-dimensional volume distribution of radiation around radioactive sources placed in the uterus and vagina. Although the theoretical dispositions have been fairly completely studied, wide variations from these physical dispositions occur both in the uterus and vagina. For example, in the uterus, the length of the organ, presence of fibroids, distortion by carcinoma, lateral deviation, acute anteversion or retroversion, will affect the dose delivered; while the relative positions of the organs may be affected by the presence or absence of packing, the degree of distension of the bladder and rectum, and the posture of the patient. Regression of a large tumor will distort the organs, while mechanical faults due to slipping of the applicator may cause serious changes in the distribution of radiation. One problem is how to correlate x-radiation with the already delivered radium dose. The only satisfactory solution is to work out the distribution of radiation due to the radium in the actual position during the treatment, and then attempt so to place the x-ray fields to make up the deficiencies in the appropriate volumes. The author discusses in detail the distributions of radiation due to 4 and 6 x-ray fields, insists on the necessity for individual detailed study of each patient, and indicates the practical methods of carrying out these investigations. The fullest cooperation between gynecologist and x-ray therapist should be undertaken before any treatment is given. Before the insertion of the radium, means should be taken to ascertain the limits of the primary growth and the extent of spread. After a full clinical assessment the treatment plan must be decided upon. The radium having been inserted, 2-plane x-ray photographs should be taken to check the correctness of the insertion, and as a record for dose estimation. Variation in the position of the radium during treatment must be investigated, and the dose distribution analyzed. Serial biopsies for cytological analysis and guidance for dosage should be undertaken in collaboration with a cytologist.—W. V. M.

The Effect of Deep X-rays on the Peritoneal Metastases of an Ovarian Carcinoma. Wyatt, J. *Proc. Roy. Soc. Med.,* **38**:183-184. 1945.

A case report.—W. V. M.

Roentgen Therapy in Diseases of the Blood-Forming Organs. Isaacs, R. [Chicago, Ill.] *Radiology,* **44**: 58-63. 1945.

X-ray treatment for diseases of the blood-forming organs cannot be according to a fixed plan but must be determined for the individual case. Variations in the course of the disease must be considered. Remissions may not occur for weeks after treatment. The author feels that best remissions are obtained with treatment given on successive days and discontinued when the white cell count falls to between 60,000 to 80,000 per cu. mm. Leukopenia appears to cause no permanent harm. The maturity of the cells in the blood stream and the total count are important. The basal metabolic rate indicates the degree of activity of the leukemic process and provides a guide to therapy, unless many immature cells are present. Five illustrative cases are described.—R. E. S.

Radiation Therapy in Chronic Leukemia. Clement, T. G. [Peoria, Ill.] *Illinois M. J.,* **87**:84-88. 1945.

In this general discussion the plea for a minimum of short wave radiation in the treatment of chronic leukemia is made. The author urges that more careful consideration be given each patient in endeavoring to see how little radiation need be employed in the treatment of chronic leukemia rather than in trying to determine how much radiation the patient can tolerate.—M. E. H.

Nervous System

Meningioma Obstructing the Foramen Magnum. Bennett, A. E., and Fortes, A. [Bishop Clarkson Memorial Hosp., Omaha, Neb.] *Arch. Neurol. & Psychiat.,* **53**:131-134. 1945.

There is no clear-cut clinical syndrome to facilitate an early diagnosis of neoplastic lesions in the location under discussion. Up to 1937 no postoperative recovery from meningioma obstructing the foramen magnum had been reported. The authors' compilation of about 60 reported and unreported cases of recovery, occurring since that time, shows that this tumor is not infrequent and that advances in neurosurgical management have been made. In the case reported here complete recovery followed a 2-stage operation.—M. E. H.

The Cerebrospinal Fluid Protein in Metastatic Brain Tumors. Shannon, E. W., and Morgan, C. W., Jr. [Massachusetts Gen. Hosp., Boston, Mass., and Cleveland Clin., Cleveland, Ohio] *New England J. Med.*, **231**:874-875. 1944.

The average protein content of lumbar cerebrospinal fluid in 43 cases of metastatic brain tumors was 99 mgm. per 100 cc. In 91% of the cases the value was greater than 40 mgm. (normal). The highest content was found in cases of superficial cerebral metastases; the patients with supratentorial metastases had a higher level than did those with subtentorial lesions. The protein content of lumbar cerebrospinal fluid alone does not differentiate a metastatic neoplasm from a primary brain tumor.—C. W.

Intramedullary Lesions of the Spinal Cord. Study of Sixty-Eight Consecutive Cases. Woods, W. W., and Pimenta, A. M. [Univ. of Michigan Med. Sch., Ann Arbor, Mich.] *Arch. Neurol. & Psychiat.*, **52**:383-399. 1944.

A clinicopathologic study of 68 consecutive cases of intramedullary spinal cord lesions treated surgically is presented with emphasis on the clinical postoperative results. Surgical treatment produced unexpectedly good results, perhaps because of the slow growth of many of the tumors. The best results were obtained when the tumor could be totally excised without damage to the cord. Intensive roentgen ray therapy following operation appears to be of benefit with certain gliomas of the spinal cord. Ependymoma and intramedullary dermoid offer a better prognosis than the more invasive gliomas. The surgical treatment of syringomyelia with or without roentgen ray therapy has not produced the encouraging results previously reported.—M. E. H.

Ganglion Cell Tumors of the Neck. Bosse, M. D., Nettrour, W. S., Soffel, J. A., and Rohm, J. Z. [West. Pennsylvania Hosp., Pittsburgh, Pa.] *Am. J. Surg.*, **65**:120-122. 1944.

The results in 2 patients are reported. In the first, a 5½ year old girl, a ganglioneuroma was successfully removed. In the other patient, 88 years old, there were pulmonary metastases, and the lesion, a ganglioneuroblastoma, proved fatal. There is a brief discussion of the origin of these tumors and of the microscopic differentiation between them.—W. A. B.

Female Genital Tract

Report of First 50 Cases of the Ovarian Tumor Registry Conducted by the American Gynecological Society. Novak, E. [Johns Hopkins Med. Sch., Baltimore, Md.] *Am. J. Obst. & Gynec.*, **48**:861-881. 1944.

Short protocols of the 50 cases with histological classifications of the tumors are given. Photomicrographs of sections of 20 tumors are included.—A. K.

Postmenopausal Ovarian Carcinoma. Brody, S. [Beth Moses Hosp., Brooklyn, N. Y.] *Am. J. Obst. & Gynec.*, **48**:417-421. 1944.

A case report. Radium castration for uterine bleeding suppressed endometrial activity and thus eliminated the physiological effect of ovarian activity on the uterus, but did not prevent subsequent development of bilateral papillary cystadenocarcinoma of the ovaries. Regular examinations at frequent intervals are urged for follow-up of patients who have received radium treatment for bleeding.—A. K.

Brenner Tumor of the Ovary Associated with Sarcomatous Change in Fibromyomata Uteri. Austin, B. R., and Ramsey, G. [Green Co. Memorial Hosp., Pa.] *Am. J. Obst. & Gynec.*, **48**:263-265. 1944.

Case report.—A. K.

Theca-Cell Tumors of the Ovary. McGoldrick, J. L., and Lapp, W. A. [Kings Co. Hosp., Brooklyn, N. Y.] *Am. J. Obst. & Gynec.*, **48**:409-416. 1944.

A report of four cases of theca-cell tumor of the ovary. All the growths were benign. One tumor was very large but produced no menstrual irregularity. In 3 patients operated upon, convalescence and follow-up were uneventful.—A. K.

Thecoma of the Ovary with Ascites and Hydrothorax (Meigs' Syndrome). Mendel, E. B., and Tyrone, C. [Ochsner Clin. and Touro Infirmary, New Orleans, La.] *Am. J. Obst. & Gynec.*, **48**:271-273. 1944.

A report of a benign thecoma of the ovary associated with hydrothorax and ascites. After removal of the tumor the patient recovered. The author emphasizes the importance of the differential diagnosis of this syndrome from metastatic malignant growths.—A. K.

Embryonic Carcinoma of Ovary in a Girl 13 Years of Age. Goldstein, S. [Montefiore Hosp., Pittsburgh, Pa.] *Am. J. Obst. & Gynec.*, **48**:129-132. 1944.

A report of an embryonic carcinoma and pseudomucinous cystadenoma of the ovary in a girl 13 years of age.—A. K.

The Place of Surgery in Fibroids of the Uterus. Barrett, C. W. [Augustana Hosp., Chicago, Ill.] *Am. J. Surg.*, **66**:148-156. 1944.

A review and discussion of the therapy of uterine fibroids. The author recommends surgical removal of these tumors whether large or small.—W. A. B.

Late Recurrence in Carcinoma of Fundus Uteri. Barrows, D. [Hosp. for Special Surg., New York, N. Y.] *Am. J. Obst. & Gynec.*, **48**:422-426. 1944.

The patient had received irradiation therapy for a carcinoma of the fundus of the uterus 9 and 4 years before hysterectomy, and was in good health 6 years after the operation. Radium therapy alone can control carcinoma of the corpus uteri for 5 years or more in a large percentage of cases, but the treatment of choice is hysterectomy if the patient can stand the operation.—A. K.

So-Called Endometrioma Interstitiale. With the Report of Three Possible Cases. deCarle, D. W. [Children's Hosp., San Francisco, Calif.] *West. J. Surg.*, **53**: 48-50. 1945.

The original definition of interstitial endometrioma is a "disease in which the interstitial cells of the endometrium have taken on invasive and vicarious growth beyond the normal bounds of the endometrium." The 3 cases reported present the same clinical and pathologic picture as those originally described by Goodall. The question arises whether such a group represents a separate disease entity. The 14 original cases and 2 of those reported here were originally classified with the sarcomas.—M. E. H.

Sarcoma Botryoides. Dwyer, W. A. [St. Joseph's Hosp., Paterson, N. J.] *Am. J. Obst. & Gynec.*, **48**:119-124. 1944.

A report of a fatal uterine rhabdomyosarcoma in a child 21 months of age. The tumor probably arose from a teratoma in either the bladder or uterine wall.—A. K.

The Minimal Histological Changes in Biopsies to Justify a Diagnosis of Cervical Cancer. TeLinde, R. W., and Galvin, G. [Johns Hopkins Univ. and Hosp., Baltimore, Md.] *Am. J. Obst. & Gynec.*, **48**:774-797. 1944.

A study was made of material from 16 cases in which diagnosis was made by biopsy and confirmed later at hysterectomy. One ovary was saved in each instance. Eleven of the cases are presented in detail, with photomicrographs. Early histological changes indicative of cancer of the cervix are discussed at length.—A. K.

Carcinoma of the Cervix. Stovall, W. D. [Univ. of Wisconsin Med. Sch., Madison, Wis.] *Wisconsin M. J.*, **44**: 212-216. 1945.

Biopsy is the only method by which an early diagnosis can be made. The technic of securing the specimen, the way it is handled after it is secured, and the training and experience of the pathologist determine the accuracy of the diagnosis.—M. E. H.

The Treatment of Carcinoma of the Cervix during Pregnancy. Jones, H. W., Jr., and Neil, W., Jr. [Kelly Clin., Baltimore, Md.] *Am. J. Obst. & Gynec.*, **48**:447-463. 1944.

In the treatment of carcinoma of the cervix during pregnancy, the decisions on whether to use radiation or to interrupt the pregnancy must be made individually for each case. Risks and prognosis for mother and child are discussed at length. Eleven case reports are presented, and tabulated data are included on 48 cases from the literature and 7 of the authors' patients.—A. K.

Pathology of Malignant Neoplasm of the Cervix Coincident with Pregnancy. Maino, C. R., Broders, A. C., and Mussey, R. D. [Mayo Clin., Rochester, Minn.] *Am. J. Obst. & Gynec.*, **48**:806-823. 1944.

A further report on 26 cases previously presented by Maino and Mussey (*Ibid.*, **47**:229. 1944; abstr. in *Cancer Research*, **5**:61. 1945). Of 24 patients followed, 8 were alive and free from recurrence 5 years later, a "cure" rate of 33%. Twenty-five patients had carcinoma, 1 had myxosarcoma. Of the carcinomas, 19 were squamous cell epitheliomas, 3 were adenocarcinomas, 2 were mixed, and in 2 cases the type was undetermined.

Bleeding was the primary symptom in 90% of cases. The extent or stage of the lesion corresponded in general to the duration or stage of pregnancy.—A. K.

Urinary System—Male and Female

Tumors of the Urogenital Tract in the Young. Bandler, C. G., and Roen, P. R. [New York Post-Grad. Med. Sch. and Hosp., Columbia Univ., New York, N. Y.] *Am. J. Surg.*, **65**:306-314. 1944.

The authors review a series of 43 cases seen in the past 20 years at New York Post-Graduate Hospital. They discuss in detail the frequently occurring Wilms' tumor, and also consider the rarer tumors of the adrenal, bladder, urethra, and testis. The value of irradiation given preoperatively is stressed. Irradiation should not be employed alone except in the presence of widespread metastases.—W. A. B.

Adenoma of the Kidney. Higgins, C. C. [Cleveland Clin., Cleveland, Ohio] *Am. J. Surg.*, **65**:3-14. 1944.

Six cases with initial symptoms of pain and, in 5 of the cases, palpable abdominal mass and hematuria are reported. All the tumors were successfully removed. A review of the literature shows 29 previously reported cases of large adenoma of the kidney.—W. A. B.

Gastrointestinal Tract

Benign Tumors of the Stomach. Dewey, E. B. [Huntington Memorial Hosp., Pasadena, Calif.] *Am. J. Surg.*, **65**:233-237. 1944.

A report of 2 cases, with a discussion of diagnosis and frequent complications.—W. A. B.

Gastric Ulcer, Benign or Malignant. Wiley, H. M. [Ellis Fischel State Cancer Hosp., Columbia, Mo.] *Am. J. Surg.*, **65**:104-111. 1944.

A review of the recent literature and a report of 2 cases in which the diagnosis of malignant growth was made only at operation.—W. A. B.

Carcinoma of the Umbilicus Metastatic from Carcinoma of the Stomach. Lombardi, L. E., and Parsons, L. [Reno, Nevada] *Ann. Int. Med.*, **22**:290-293. 1945.

Case report.—J. G. K.

Carcinoma of the Small Intestine. McDougal, W. J. [Butterworth Hosp., Grand Rapids, Mich.] *Am. J. Surg.*, **66**: 119-122. 1944.

A discussion of the symptomatology and diagnosis of carcinoma of the small bowel, with a case report.—W. A. B.

Lymphangioma of the Jejunum. Puppel, I. D., and Morris, L. E., Jr. [Ohio State Univ., Columbus, Ohio] *Arch. Path.*, **38**:410-412. 1944.

Report of a case in a woman of 55 years. Weakness, anemia, and melena were present before resection of the lymphangioma.—J. G. K.

Adenocarcinoma of the Ileum in a Girl of Thirteen. Puppel, I. D., and Morris, L. E., Jr. [Ohio State Univ., Columbus, Ohio] *Am. J. Surg.*, **66**:113-115. 1944.

Report of a case. The child had had symptoms of intermittent obstruction for one year before operation. Operation was occasioned by an episode of intussusception, and the tumor was completely removed. There was no evidence of metastasis, and the patient was in excellent health one year later.—W. A. B.

Case Reports of Barnes Hospital. Clinical and Post Mortem Records Used in Weekly Clinicopathologic Conferences at Barnes Hospital, St. Louis. Case 58. Wood, W. B., Jr., and Moore, R. A., Editors. [St. Louis, Mo.] *J. Missouri M. A.*, **41**:243-247. 1944.

A report of a patient with 2 primary tumors, *i.e.*, primary carcinoma of the stomach and primary carcinoma of the ascending colon.—M. E. H.

Carcinoma simulating Appendicitis. Roberts, A. [Runwell Hosp., Wickford, England] *Brit. M. J.*, **i**:153. 1945.

Metastases, involving the appendix and derived from a small rectosigmoid carcinoma, produced a clinical picture like that of acute appendicitis.—E. L. K.

The Association of Squamous Cancer With Anal Manifestations of Lymphogranuloma Venereum. Binkley, G. E., and Derrick, W. A. [New York, N. Y.] *Am. J. Digest. Dis.*, **12**:46-47. 1945.

The incidence of anal cancer in patients with chronic anal manifestations of lymphogranuloma venereum suggests that the latter may be a predisposing factor. Frei tests for lymphogranuloma venereum, made on 19 patients with squamous anal and rectal cancer at Memorial Hospital, New York, gave 8 positive results. Many of the remaining 11 patients gave evidence strongly suggesting pre-existing anal lymphogranuloma venereum.—R. E. S.

Bone and Bursa

Osteoid Osteoma of Midshaft Region of Femur. Harmon, P. W. [Guthrie Clin., and Robert Packer Hosp., Sayre, Pa.] *Am. J. Surg.*, **66**:128-131. 1944.

A summary of the recent literature, and report of a case of osteoid-osteoma in a 9 year old girl, whose only symptom was pain for 10 months before operative removal of the lesion.—W. A. B.

Epidermoid (Squamous Epithelial) Bone Cyst of Phalanx. Pohlmann, H. F., and Wachstein, M. [Elizabeth A. Horton Memorial Hosp., Middletown, N. Y.] *Ann. Surg.*, **119**:148-154. 1944.

A case of epidermoid bone cyst treated by excision followed by electric cauterization of the cavity is reported, together with a review of 10 similar cases from the literature and a discussion of the differential diagnosis.—W. J. B.

Chondrosarcoma of a Phalanx with Cutaneous Metastases. Cruickshank, A. H. [Aberdeen Univ., Aberdeen, Scotland] *J. Path. & Bact.*, **57**:144-145. 1945.

A case of chondrosarcoma of the middle phalanx of the left middle finger occurring in a male subject aged 66 is described. This appeared histologically to be simple, but radiologically there was a suspicion of malignancy. Two years after amputation of the affected finger, multiple metastases developed in the skin of the face and in the lungs.

The author records that the metastases were apparently benign on histological examination and draws attention to the necessity for revising the traditional opinion that phalangeal chondroma is always benign.—L. W. P.

Cystic Tumor of the Ileopectineal Bursa. Report of Two Cases. Stephens, V. R. [Univ. of Illinois Coll. of Med., and MacNeal Memorial Hosp., Berwyn, Ill.] *Arch. Surg.*, **49**:9-11. 1944.

These cystic tumors occurred in elderly men whose occupation involved strenuous use of the legs. In one case there was no connection between the bursa and the hip joint, which was normal; in the other there was a wide communication, and arthritic and atrophic changes were present in the joint.—W. A. B.

Miscellaneous

Primary Carcinoma of the Uterus and a Primary Carcinoma of the Breast in the Same Individual Twenty-Three Years Later. Lincoln, W. A. [Calgary, Canada] *Canad. M. A. J.*, **50**:445-446. 1944.

Case report.—M. E. H.

Unusual Lipoma. McGill, J. J. [Denver, Colo.] *Rocky Mountain M. J.*, **42**:111-112. 1945.

A report of a case of benign lipoma of the buttock.—M. E. H.

Case Reports of Barnes Hospital. Clinical and Postmortem Records Used in Weekly Clinicopathologic Conferences at Barnes Hospital, St. Louis. Wood, W. B., Jr., and Moore, R. A. [Barnes Hosp., St. Louis, Mo.] *J. Missouri M. A.*, **42**:93-103. 1945.

In Case 61 there was a carcinoma of the apex of the right lung with extension to the right parietal pleura and to the mediastinum, and metastases to all lobes of the lungs, to the bronchopulmonary and tracheobronchial lymph nodes, and to the right lobe of the liver. The carcinoma compressed the lumen and invaded the wall of the superior vena cava; it also compressed the pulmonary artery to the upper lobe of the right lung. Case 62 was an example of carcinoma of the sigmoid colon, with dilatation of the large and small intestines above the carcinoma, acute and chronic ulcerative colitis, and acute ulcerative enteritis.—M. E. H.

Statistics

Statistical Analysis of 2,407 Admissions to the Tumor Clinic of Hines Veterans Hospital, During 1943. Schrek, R., and Allaben, G. R. [Veterans Admin., Hines, Ill.] *Cancer Research*, **5**:539-546. 1945.

During 1943 the tumor clinic admitted 1,623 patients for the first time, and readmitted 581 patients. This report presents a detailed analysis of the racial, geographic, and age distribution of the patients with various types of neoplasia. The type of treatment administered and the number of deaths in the hospital are also considered. A relatively high percentage (51%) of the patients with carcinoma of the exposed skin came from southern states, but low percentages (17 and 12%) of the patients with cancer of the stomach and testis were also from the South. Relatively many patients with carcinoma of the exposed skin and lip, but few with glioma of the brain, came from rural districts. Carcinoma of the exposed skin, lip, and larynx occurred relatively more often in white than in colored patients, but carcinoma of the esophagus occurred relatively more often in the colored. The average age of the patients was 50 years, but those with cancer of the testis, glioma of the brain, or Hodgkin's disease had a lower average age (36 to 41 years). The mortality rate for all patients was 24%. Carcinoma of the esophagus had the highest percentage of deaths (63) and carcinoma of the exposed skin the lowest (1.3).—Authors' abstract.